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A REVIEW OF ECOLOGICAL ASSESSMENT CASE STUDIES

FROM A RISK ASSESSMENT PERSPECTIVE

VOLUME II

Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC 20460

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FOREWORD

Since 1990, the Risk Assessment Forum of the U.S. Environmental Protection Agency (EPA) has sponsored activities to improve the quality and consistency of EPA's ecological risk assessments. Projects have included development of Agencywide guidance on basic ecological risk assessment principles (Framework Report, U.S. EPA, 1992) and evaluation of 12 ecological assessment case studies from a risk perspective (U.S. EPA, 1993). To complement this original set of case studies, several new case studies were recently evaluated to provide further insight into the ecological risk assessment process.

As with the original case studies, each of the five new case studies was evaluated by scientific experts at EPA-sponsored workshops. Two workshops were held in September 1992 (57 Federal Register 38504, August 25, 1992); these workshops were chaired by Dr. Charles Menzie and included reviewers from universities, private organizations, and industry.

The new case studies expand the range of the first case study set by including different kinds of stressors (radionuclides, genetically engineered organisms, and physical alteration of wetlands) and programmatic approaches (premanufacture notice assessments under the Toxic Substances Control Act and the EPA's Environmental Monitoring and Assessment Program). In addition, the authors and reviewers of the new case studies were able to use EPA's Framework Report as background information. Both sets of case studies provide useful perspectives concerning application of ecological risk assessment principles to "real world" problems.

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SUMMARY

As with the previous case studies report (U.S. EPA, 1993), this document uses case studies to explore the relationship between the ecological risk assessment process and approaches used by EPA (and others) to evaluate adverse ecological effects. In contrast to the earlier report, the authors and reviewers of these case studies were able to use EPA's *Framework for Ecological Risk Assessment* (Framework Report, U.S. EPA, 1992) as background information. However, even though the case studies have been structured as described in the Framework Report, most were not originally planned and conducted as risk assessments. This should be kept in mind when considering each case study's strengths and limitations.

Some of the contributions of the case studies in this report to a broader understanding of the ecological risk assessment process are highlighted below.

- The application of the framework approach to nonchemical stressors is explored. Examples include biological stressors (genetically engineered microorganisms), physical stressors (alteration of wetland function by a variety of physical disturbances), and radioactivity (radionuclides in water).

- The relationship of ecological risk assessment to a major EPA monitoring program (Environmental Monitoring and Assessment Program—EMAP) is described.

- Regional scale assessments (EMAP, wetlands) are included.

- Conducting an ecological risk assessment in a tiered fashion starting with minimal exposure and effects data is illustrated by the premanufacture notice (PMN) review carried out under the Toxic Substances Control Act.

While these cases are representative of the state of the practice in ecological assessments, they should not be regarded as models to be followed. Rather, they should be used to attain a better understanding of ecological risk assessment practices and principles. These case studies and others being prepared will be used along with the Framework Report to provide a foundation for future Agencywide guidelines for ecological risk assessment.

PART I. CASE STUDIES OVERVIEW

1. INTRODUCTION

In 1990, the Risk Assessment Forum initiated an effort to develop Agencywide guidance for conducting ecological risk assessments. This effort consists of several parts, as described below.

- Basic principles and terminology for ecological risk assessment are described in the report *Framework for Ecological Risk Assessment* (Framework Report) that was published in 1992 (U.S. EPA, 1992).

- Scientific/technical background information for development of future EPA ecological risk assessment guidelines will be contained in a series of issue papers based on the Framework Report that are now in preparation.

- Case studies are being developed to provide "real world" examples of how ecological risk assessments can be conducted. The first set of 12 case studies has been published (U.S. EPA, 1993).

This report includes five additional case studies that have been peer-reviewed and organized according to the ecological risk assessment process as described in the Framework Report. As with the first case studies report, this document should be useful to EPA regional, laboratory, and headquarters personnel conducting ecological risk assessments, as well as to interested individuals from other federal and state agencies and the general public. The Forum plans to continue development of other case studies as a means of illustrating the application of ecological risk assessment principles.

2. GUIDE TO THE CASE STUDIES

2.1. Background

The case studies presented in part II of this report illustrate several types of ecological assessments. As summarized in table 1, these cases involve:

- studies done under several different federal environmental laws;
- spatial scales ranging from local impacts to national impacts;
- different types of stressors (chemical, physical, and biological);
- a variety of ecosystems, including aquatic (freshwater and marine), wetlands, and terrestrial; and
- measurement endpoints reflecting different levels of biological organization, ranging from effects on individual organisms up to and including effects on ecosystems (see part I, section 3, for definitions of measurement and assessment endpoints).

These case studies expand the range of the first case study set (U.S. EPA, 1993) by including different kinds of stressors (radionuclides, genetically-engineered organisms, and physical alteration of wetlands) and programmatic approaches (Pre-Manufacture Notice assessments under the Toxic Substances Control Act and the EPA's Environmental Monitoring and Assessment Program).

2.2. Case Study Highlights

This section highlights some common themes and principles gleaned through development and review of these case studies. This section is organized according to the framework for ecological risk assessment provided in the recently published Framework Report (U.S. EPA, 1992) (see figure 1):

- **Problem formulation**, which is a preliminary scoping process;
- **Analysis**, which includes characterization of both ecological effects and exposure; and
- **Risk characterization**, which highlights qualitative and quantitative conclusions, with special emphasis on data limitations and other uncertainties.

Table 1. Case Study Characteristics

No.[a]	Short Title	Relevant Federal Legislation[b]	Spatial Scale of Assessment	Stressor Type[c]	Ecosystem Type[d]	Level of Biological Organization[e]
1	New Chemical	TSCA	National	SC	A/F	Individual
2	Recombinant Rhizobia	TSCA	Local	B	T	Individual
3	Radionuclides	CERCLA/SARA, CWA	Local	CM	A/F	Individual
4	Wetlands	CWA, EWRA	Regional	P, CM	W, A/F	Ecosystem
5	EMAP	--	Regional	P, CM	A/M	Community

[a] Numbers 1-5 refer to the sections of part II of this report.

[b] Legislation

CERCLA/SARA: Comprehensive Environmental Response, Compensation, and Liability Act (1980)/ Superfund Amendments and Reauthorization Act (1987)
CWA: Clean Water Act (1977)
EWRA: Emergency Wetlands Resources Act (1986)
TSCA: Toxic Substances Control Act (1976)

[c] Stressor types

B: Biological
MC: Mixture of chemicals
P: Physical stressor
SC: Single chemical

[d] Ecosystem types

A/F: Aquatic—freshwater
A/M: Aquatic—marine or estuarine
T: Terrestrial
W: Wetlands

[e] Highest level of biological organization for the measurement endpoints used.

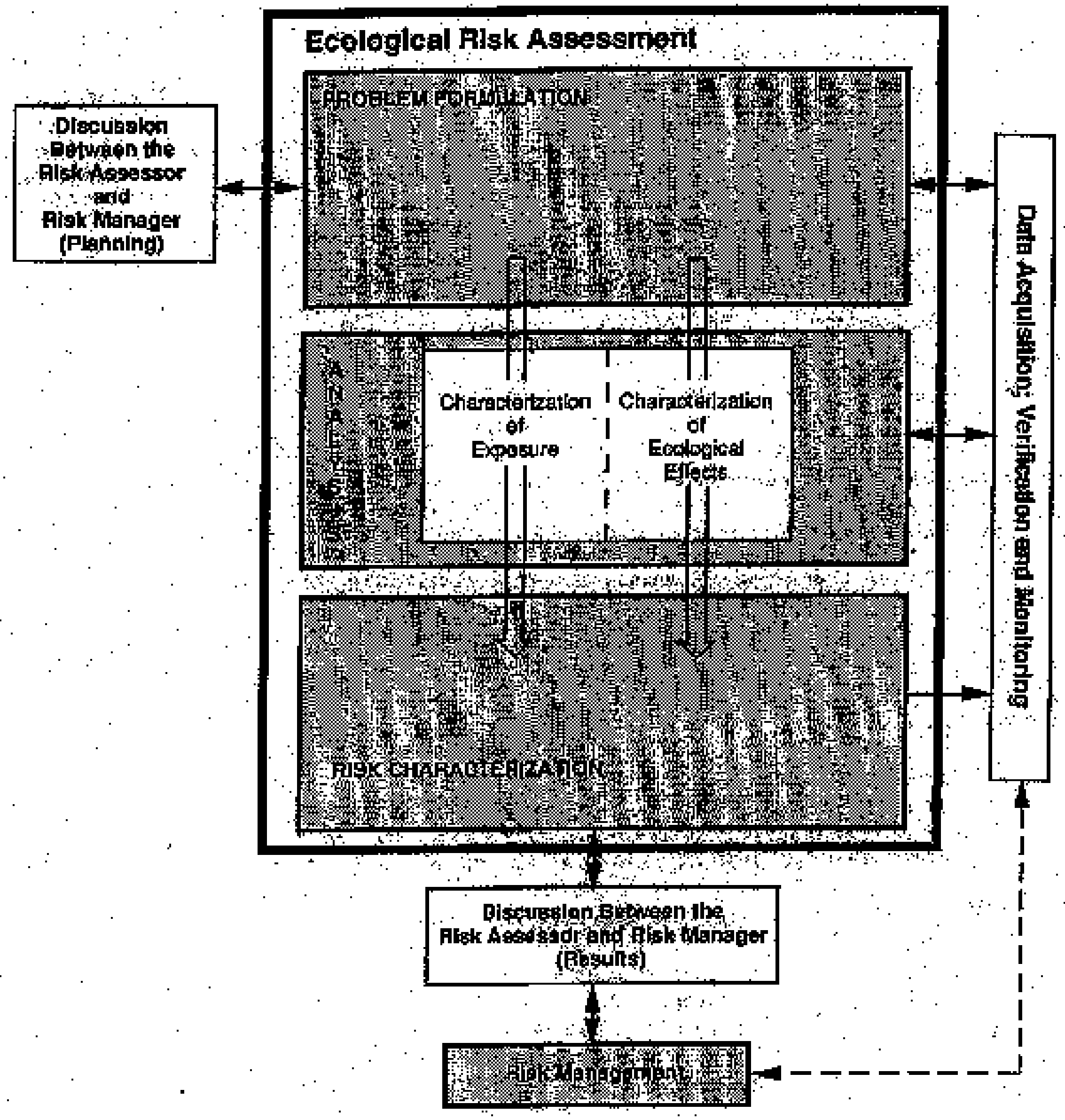

Figure 1. **The framework for ecological risk assessment (U.S. EPA, 1992). The ecological risk assessment framework is the product of a series of workshops and reviews that involved both EPA and outside scientists. While the Framework Report has been a critical first step in developing ecological risk assessment concepts, evolution of the framework concepts is expected and encouraged.**

2.2.1. Problem Formulation

Problem formulation is an initial planning and scoping process for defining the feasibility, breadth, and objectives for the ecological risk assessment. The process includes preliminary evaluation of exposure and effects as well as examination of scientific data and data needs, regulatory issues, and site-specific factors. Problem formulation defines the ecosystems potentially at risk, the stressors, and the measurement and assessment endpoints. This information then may be summarized in a conceptual model, which hypothesizes how the stressor may affect the ecological components (i.e., the individuals, populations, communities, or ecosystems of concern).

Two of the most important themes that emerged from a review of the 12 case studies (U.S. EPA, 1993) and that were clearly evident in the review of the five case studies presented in this document are as follows:

- Thorough formulation of the problem and development of the scope are essential first steps for a successful risk assessment.

- It is important to clearly articulate management issues at the beginning of an assessment.

The strengths and limitations of the case studies often were related to the care taken in formulating the problem and articulating management issues at the beginning of the assessment. Examples in this set of case studies that demonstrate careful implementation of these steps include the New Chemical and Radionuclides case studies.

Monitoring Programs Can Provide Data Useful for Problem Formulation

The EMAP case study was unique in that it illustrated how monitoring data can be used at the problem formulation stage of an assessment. As indicated in figure 1, data acquisition, verification, and monitoring provide information that supports all phases of ecological risk assessment. The EMAP Near Coastal program in the Virginia Biogeographic Province is an example of a provincewide monitoring program in which data are collected using a systematic, probability-based design that facilitates detection of spatially distributed events but does not estimate intraannual variability or short-term episodic events.

The monitoring program obtains data throughout the province on a variety of exposure and effects indicators. The indicators were chosen based on past monitoring experience with regard to environmental conditions in coastal systems. Associations between exposure and effects indicators imply neither causality nor direct effects from anthropogenic stressors. As noted in the EMAP case study, "It is important to recognize that monitoring data alone will not be sufficient for establishing the causal relationships necessary for developing a complete analysis of ecological risk." Taken along with other evidence, however, associations between exposure and effects indicators can be used to direct further study and to aid in problem formulation.

The EMAP case study also illustrates how information obtained from provincewide monitoring can be used in the problem formulation phase for more local or regional risk assessments. The monitoring tools and the design employed within EMAP can be applied to these smaller spatial scales.

The Framework Can Be Applied to Such Diverse

The previous review of 12 case studies (U.S. EPA, 1993) indicated that the framework can be applied to chemical and physical stressors. This

Stressors as Radionuclides and Genetically Engineered Organisms

was demonstrated further with the present set of five case studies, which includes assessments of the environmental release of a new chemical substance and physical modifications of wetlands. The Radionuclides case study showed that the framework is applicable to radionuclides as well as to hazardous chemicals.

The authors and reviewers of the case study on the release of recombinant rhizobia, a genetically engineered organism, concluded that application of the framework to microbial stressors is possible. It was generally agreed, however, that the unique properties and complexities of a living, changing stressor should be acknowledged in the framework and in subsequent case studies with a similar focus. Stressors potentially associated with the rhizobia were characterized as either biological (i.e., pathogenicity, altered legume growth, microbial competition, and gene release) or chemical (i.e., toxins and detrimental metabolites); the reviewers of the case study found this to be a useful approach. The case study author found it difficult to select endpoints and to decide whether these represented assessment or measurement endpoints.

Iterative Approaches Are Useful for Defining Problems and Allocating Resources

As noted in the Framework Report (U.S. EPA, 1992), ecological risk assessments are frequently iterative, with data collection and analysis performed in tiers of increasing complexity and cost. The New Chemical case study illustrates this process. Ecological risk assessments are conducted for new chemical substances under the Toxic Substances Control Act in EPA's Office of Pollution Prevention and Toxics (OPPT). In these assessments, there is a progression from a simple screening approach to more resource-intensive evaluations based on the results of the simpler analysis, consideration of associated uncertainties, and identification of data gaps. The authors note that because of the large number of PMNs received annually by OPPT, the only practical approach is to use conservative screening estimates initially and to proceed to more detailed assessments only when necessary.

All Important Exposure Scenarios Should Be Considered

Exposure routes should be carefully considered during problem formulation to ensure that the risk assessment is properly focused. For example, in two of the case studies, the reviewers suggested that additional routes of exposure could have been included in the risk assessments. In the New Chemical case study, exposure to suspended sediments was suggested, while in the Radionuclides case study, potential uptake from food could have been evaluated in addition to direct uptake from water.

2.2.2. Analysis

Analysis includes the technical evaluation of data on both potential exposure to stressors (characterization of exposure) and the effects of stressors (characterization of ecological effects). Characterizing exposure involves predicting or measuring the spatial and temporal distribution of a stressor and its co-occurrence, or contact, with the ecological components of concern; characterizing ecological effects involves identifying and quantifying the effects elicited by a stressor and, to the extent possible, evaluating cause-and-effect relationships.

2.2.2.1. Characterization of Exposure

Models Provided Useful Tools for Characterizing Exposure

As with the previous compendium of case studies, this set demonstrates that simple as well as more complex models can help to characterize the exposure field. Selection of models should be based on the goals of the assessment as well as the availability of data and resources. In the New Chemical case study, a simple dilution model was initially used to estimate exposure concentrations in receiving water. Based on the results from this model, which showed that exposures could result in risk to aquatic organisms, a more complex model was used to provide a more accurate but less conservative estimate of exposure.

"Reality Checks" Are Important for Exposure Estimates Based on Models

While exposure models can be useful, some degree of model verification is important to reduce uncertainty. The Radionuclides case study used a bioaccumulation model to estimate dose. When the predicted doses were checked against a set of measurements, the model was found to be conservative in some respects. The reviewers observed that exposure may not be reliably predicted from radionuclide activity in water, given the high variance found in bioconcentration factors. In the Recombinant Rhizobia case study, field measurements conducted after the risk assessment was completed verified the literature-based predictions concerning off-site migration of the rhizobia microorganisms.

Evaluating Exposure to Genetically Engineered Organisms Poses Special Problems

Biological stressors were not addressed in the Framework Report, but are the subject of the Recombinant Rhizobia case study, which highlights some of the difficulties associated with predicting and monitoring the spread of a stressor that is a living organism. Exposure evaluation is most challenging because of the organism's capacity to interact with its environment and to evolve. Moreover, because it is capable of growth and reproduction, the stressor can increase in amount over time as compared with amounts of chemical stressors, which are either conservative or decrease with time and/or distance from sources.

2.2.2.2. Characterization of Ecological Effects

Effects Information Is Developed From Predictive Methods, Literature Values, Laboratory Studies, and Field Programs

The case studies demonstrate the range in sources of information used for characterizing ecological risks. The Radionuclides case study and the Wetlands case study relied primarily on existing guidelines or literature values to characterize effects. The potential effects of rhizobia were based on greenhouse studies, while the EMAP case study used a suite of field studies. The New Chemical case study utilized quantitative structure-activity relationships (QSARs) based on molecular weight and log K_{ow} as one source of information concerning toxicity. QSAR methods were particularly useful in this application given the large number of PMNs that need to be evaluated by EPA. This case study also relied on laboratory bioassays. The author noted that larger-scale studies (e.g., of mesocosms) have not been used routinely because of cost considerations. Nonetheless, OPPT is initiating field mesocosm studies to evaluate the use of laboratory tests for predicting effects in the field.

Most Effects Information Is Developed for

Most of the effects information presented in the case studies is based on small groups of organisms tested as individual species. Because effects data on mortality, growth, and reproduction are developed for the individual, there is a general lack of information on effects at the

Individual Organisms in Single- Species Tests

population level. Assessment endpoints, however, often are expressed in terms of populations or communities of organisms. Similarly, data from single species of organisms are used to derive stressor levels that will be protective of communities or ecosystems, without consideration of indirect effects or interspecies interactions. The use of such extrapolations is a continuing area of controversy and discussion in ecological risk assessment.

Multiple Stressors Complicate Evaluations of Causality

Individual stressors do not occur in a vacuum in the real world. Rather, accompanying the stressor of interest may be a host of other chemical, biological, or physical stressors that may alter or confound the effects and risks associated with the subject stressor. Thus the EMAP case study noted that results of monitoring do not necessarily indicate causality. Reviewers of the New Chemical case study noted that the effects of the chemical could depend on the presence of other chemicals in a complex effluent. While the Radionuclide case study concluded that radionuclides posed little risk to important fish species in the Columbia River, the limited scope of the case precluded consideration of other chemical and physical stressors that may pose a much higher risk to fish populations. The Wetlands case study examined the effects on wetland water quality status of a range of stressors, including physical and hydrologic disturbances and loss or conversion of wetland habitat. Several stressors were present at most of the study sites. A multiple regression approach was used to relate the effects of different stressors to water quality impacts.

2.2.3. Risk Characterization

Risk characterization uses the results of the exposure and ecological effects analyses to evaluate the likelihood that adverse ecological effects are occurring or will occur in association with exposure to a stressor. Essentially, a risk characterization highlights summaries of the assumptions, scientific uncertainties, and strengths and weaknesses of the analyses. Additionally, a risk characterization evaluates the ecological significance of the risks with consideration of the types and magnitudes of the effects, their spatial and temporal patterns, and the likelihood of recovery.

Most of the Case Studies Used the Quotient Method to Integrate Exposure and Effects Estimates

The Quotient Method was used in three of the five case studies: New Chemical, Wetlands, and Radionuclides. While the Quotient Method does not measure risk in terms of a likelihood of effects at the individual or population level, it does provide a simple benchmark for judging risk potential. As such, it has been widely used. The most common application of the Quotient Method in aquatic ecological risk assessments is to compare an estimate of a maximum exposure concentration to a water quality criterion for a chemical. While reliance on the Quotient Method in the present set of case studies is consistent with the previous set of 12 case studies (U.S. EPA, 1993), development and use of other ecological risk integration techniques that can provide actual risk estimates should be encouraged. When the Quotient Method is used, at least a qualitative description of key study uncertainties and limitations should be provided.

Risks to Populations Were Qualitatively Discussed

Both the previous and present set of case studies made only limited attempts at directly estimating population-level risks. Typically, risks are assessed at the individual level, and population-level risks then are inferred from the presence of risks to individuals. It is indeed probable that when estimates indicate little or no risk to individuals, there is little or no risk to the population. However, when there are risks to individuals, there may or may not be risks to the population. Thus the extrapolation from risks to individuals to risks to populations is frequently discussed as an area of uncertainty within the risk assessments.

Stressor-Response Models Are Useful in Both Predictive and Retrospective Assessments

The previous set of risk assessments (U.S. EPA, 1993) illustrated the value of stressor-response models in quantitative risk assessment. In the present set, the Wetlands case study used regression techniques to develop stressor-response models for water quality impacts resulting from a wide range of physical stressors. The reviewers of this case study noted that this empirical statistical model was a key feature of the case study and provided a predictive component. However, because this model is based on a particular set of physical and hydrological characteristics, predictions of the model may or may not be representative of other urban wetlands.

The EMAP case study was retrospective in nature because it examined the relationship between indicators of the status of ecological resources and an array of stressors. Although this case study was not a risk assessment, it clearly showed that an understanding of stressor-response relationships would be an important component of any future risk assessment that evaluated the causal links between sources, stressors, and observed effects.

Major Sources of Uncertainty Should Be Identified

Uncertainties associated with the use of available data for risk assessments were mentioned in most of the case studies. The New Chemical case study described the use of fixed "assessment factors" to deal with extrapolations between different types of data. The EMAP case study cautioned against assuming causality based on apparent associations derived from monitoring exposure and effects indicators. The authors and reviewers of the case studies frequently pointed out potential problems in extrapolating between species and from the laboratory to the field, in accounting for the combined effects of multiple stressors, and in interpreting the results of field tests. Although it is important to identify the major sources of uncertainty in a risk assessment, the presence of uncertainty does not necessarily preclude use of the risk assessment for risk management decisions.

A Weight-of-Evidence Approach Can Be Useful in Risk Assessments

The availability of multiples sources of information can help to strengthen a risk estimate even when individual lines of evidence are not conclusive. For example, in the Recombinant Rhizobia case study, the reviewers felt that the data from the greenhouse studies and field tests by themselves were not convincing. However, the availability of information characterizing the rhizobia strains and documenting the effects of previous releases of other rhizobia helped strengthen the overall risk assessment conclusion that the small-scale field test of the recombinant rhizobia should proceed. The EMAP case study also uses a weight-of-evidence approach in problem formulation (not risk characterization). Stressor and effects information derived from the monitoring program are used to identify areas of greatest concern that may be candidates for ecological risk assessment.

3. KEY TERMS (U.S. EPA, 1992)

assessment endpoint—An explicit expression of the environmental value that is to be protected.

characterization of ecological effects—A portion of the analysis phase of ecological risk assessment that evaluates the ability of a stressor to cause adverse effects under a particular set of circumstances.

characterization of exposure—A portion of the analysis phase of ecological risk assessment that evaluates the interaction of the stressor with one or more ecological components. Exposure can be expressed as co-occurrence or contact, depending on the stressor and ecological component involved.

conceptual model—The conceptual model describes a series of working hypotheses of how the stressor might affect ecological components. The conceptual model also describes the ecosystem potentially at risk, the relationship between measurement and assessment endpoints, and exposure scenarios.

ecological component—Any part of an ecological system, including individuals, populations, communities, and the ecosystem itself.

ecological risk assessment—The process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors.

exposure—Co-occurrence of or contact between a stressor and an ecological component.

measurement endpoint—A measurable ecological characteristic that is related to the valued characteristic chosen as the assessment endpoint. Measurement endpoints are often expressed as the statistical or arithmetic summaries of the observations that comprise the measurement.

risk characterization—A phase of ecological risk assessment that integrates the results of the exposure and ecological effects analyses to evaluate the likelihood of adverse ecological effects associated with exposure to a stressor. The ecological significance of the adverse effects is discussed, including consideration of the types and magnitudes of the effects, their spatial and temporal patterns, and the likelihood of recovery.

stressor—Any physical, chemical, or biological entity that can induce an adverse response.

4. REFERENCES

U.S. Environmental Protection Agency. (1992) *Framework for ecological risk assessment*. Risk Assessment Forum, Washington, DC. EPA 630/R-92/001.

U.S. Environmental Protection Agency. (1993) *A review of ecological assessment case studies from a risk assessment perspective.* Risk Assessment Forum, Washington, DC. EPA/630/R-92/005.

PART II. THE CASE STUDIES

Authors of the case studies included in this section were asked to follow the format shown in the box on the right. As you read the case studies, it is important to keep several points in mind:

> **Case Study Format**
>
> - Abstract. The abstract summarizes the major conclusions, strengths, and limitations of the case study.
> - Risk Assessment Approach. This section clarifies any differences between the ecological risk assessment approach used in the case study and the general process described in the Framework Report.
> - Statutory and Regulatory Background. The statutory requirements for the study are described along with any pertinent regulatory background information.
> - Case Study Description. This contains the background information and objective for the case study, followed by the technical information organized according to the ecological risk assessment framework: problem formulation, analysis (characterization of exposure and characterization of ecological effects), and risk characterization. A comment box is included at the end of each major section.
> - References.

- **The original case studies were not necessarily developed as risk assessments as defined in the Framework Report**. EPA notes that the case studies are often partial risk assessments that focus on available information without discussing other relevant considerations such as the uncertainties defined by a limited data base.

  At the workshops, each case study was evaluated as to whether it (1) effectively addressed the generally accepted components of an ecological risk assessment, or (2) addressed some but not all of these components or, instead, (3) provided an alternative approach to assessing ecological effects.

- **The strengths and limitations of each case study are highlighted in comment boxes at the end of the problem formulation, analysis, and risk characterization sections**. Author's comments address issues raised in the preceding text or reviewer remarks from the peer review of the case study. Reviewers' comments include strengths, limitations, and general observations concerning the case studies.

- **The authors who compiled the case studies did not necessarily conduct the research upon which the case studies are based**. References to the original research are provided in each case study.

The general characteristics of the case studies are summarized in table 1 (in part I). Case studies are referenced by the section of this report in which they appear. (The corresponding short titles of the case studies are given in table 1).

SECTION ONE

ECOLOGICAL RISK ASSESSMENT CASE STUDY:

ASSESSING THE ECOLOGICAL RISKS OF A NEW CHEMICAL UNDER THE TOXIC SUBSTANCES CONTROL ACT

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LIST OF ACRONYMS

CBI	confidential business information
CC	concern concentration
ChV	chronic value
CSRAD	Chemical Screening and Risk Assessment Division
EC_{50}	median effect concentration
EEB	Environmental Effects Branch
EETD	Economics, Exposure and Technology Division
EXAMS II	exposure analysis modeling system
HERD	Health and Environmental Review Division
K_{oc}	soil/sediment organic carbon-water partition coefficient
K_{ow}	octanol-water partition coefficient
LC_{50}	median lethal concentration
MATC	maximum acceptable toxicant concentration
OPPT	Office of Pollution Prevention and Toxics
PDM3	probabilistic dilution model
PEC	predicted environmental concentration
PMN	premanufacture notice
QSAR	quantitative structure-activity relationship
SAR	structure activity relationship
SNUR	significant new use rule
POTW	publicly owned treatment works

ABSTRACT

This case study is an example of how the Office of Pollution Prevention and Toxics (OPPT) conducts ecological risk assessments for new chemical substances. The Toxic Substances Control Act requires manufacturers and importers of new chemicals to submit a premanufacture notice (PMN) to EPA 90 days before they intend to begin manufacturing or importing. Because actual test data are not required as part of a PMN submission, EPA uses structure-activity relationships to estimate both ecological effects and exposure.

The PMN substance is a neutral organic compound. This class of compounds elicits a simple form of toxicity known as narcosis. The toxicity of neutral organic compounds can be estimated through quantitative structure-activity relationships, which correlate toxicity with molecular weight and the octanol-water partition coefficient (log K_{ow}). The subject PMN substance has a log K_{ow} of 6.7. Compounds with such a log K_{ow} are not expected to be acutely toxic (no effects at saturation over short exposure durations) but are expected to elicit chronic effects. Actual testing of the PMN substance confirmed these predictions.

The manufacturer identified processing, use, and disposal sites adjacent to rivers and streams. Because it was expected that the PMN substance would be discharged to such environments, pelagic and benthic aquatic populations and communities were considered to be potentially at risk. Therefore, the assessment endpoint used in this case study was the protection of aquatic organisms (e.g., algae, aquatic invertebrates, and fish). Measurement endpoints used to evaluate the risks to the assessment endpoint were mortality, growth and development, and reproduction.

Initial exposure concentrations were estimated using a simple dilution model that divided releases (kg/day) by stream flow (millions of liters/day). Subsequent exposure analyses used a probabilistic dilution model (PDM3) and the exposure analysis modeling system (EXAMS II). PDM3 was used to estimate the number of days a particular effect concentration would be exceeded in 1 year, and EXAMS II was used to estimate concentrations in the water column and sediments using generic site data.

In risk characterization, the quotient method was used to compare exposure concentrations with ecological effect concentrations. A ratio of 1 or greater indicates a risk. The case study presents five iterations of analysis and risk characterization. The first four iterations identified an ecological risk and resulted in the collection of additional ecological effects test data and more information on potential exposure to the PMN substance. The final outcome was that the PMN substance could be used only at the identified sites because there was uncertainty as to whether the concern level (1 µg/L) might be exceeded at sites not identified by the manufacturer.

OPPT terminology differs from terminology in EPA's *Framework for Ecological Risk Assessment* (Framework Report; U.S. EPA, 1992). For example, OPPT uses "Hazard Assessment" instead of "Characterization of Ecological Effects." Otherwise, the OPPT ecological risk assessment procedure follows the approaches and concepts described in the first- and second-order diagrams of the Framework Report.

1.1. RISK ASSESSMENT APPROACH

This case study follows EPA's Framework Report (figure 1-1); that is, it is composed of three phases: problem formulation, analysis, and risk characterization.

The Office of Pollution Prevention and Toxics' (OPPT's) overall approach to assessing the risks of new chemicals is to compare exposure concentrations with ecological effect concentrations. The process often begins with simple stream flow dilution models that typically result in a worst-case scenario. If a risk is ascertained, more detailed analyses are performed (figure 1-2). Because of the paucity of data associated with premanufacture notice (PMN) submissions (see discussion under Statutory and Regulatory Background), there is a heavy reliance on the use of structure-activity relationships (SARs) to estimate ecological effects and develop a stressor-response profile.

Figure 1-2 does not include risk management options. In addition to obtaining additional exposure and ecological effects information, risk management options can include a variety of regulatory enforcement actions such as banning discharges to water or requiring pretreatment. In any event, risk assessors must ascertain that a risk exists before risk managers can exercise their management options.

The case study has the following strengths: (1) it relates measurement endpoints to an assessment endpoint; (2) it demonstrates that ecological risk assessments can be conducted with minimal ecological effect and exposure data; and (3) it demonstrates the usefulness of SARs in establishing a stressor-response profile.

One weakness of the case study is the lack of a true quantification of the effects to the assessment endpoint (populations of aquatic organisms). However, this is a weakness only from the scientific point of view; it was not needed from the regulatory point of view. Another weakness is that the risk assessors expected the PMN substance to bioconcentrate, yet they did not analyze the potential risks to predators that might ingest contaminated prey.

1.2. STATUTORY AND REGULATORY BACKGROUND

The Toxic Substances Control Act (TSCA) provides for the regulation of chemicals not covered by other statutes (e.g., Food, Drug, and Cosmetic Act; Federal Insecticide, Fungicide, and Rodenticide Act). Enacted in 1976, TSCA regulates industrial chemicals such as solvents, lubricants, dyes, and surfactants. TSCA requires the assessment and, if necessary, regulation of all phases of the life cycle of industrial chemicals: manufacturing, processing, use, and disposal.

TSCA regulates two categories of industrial chemicals: (1) chemicals on the TSCA Chemical Substances Inventory List and (2) new chemicals. The TSCA Chemical Substances Inventory includes chemicals in commercial production between 1975 and 1979, and chemicals reviewed under the PMN program and commercially produced after 1979. New chemicals are those substances that do not appear on the TSCA inventory. Section 5 of TSCA requires manufacturers and importers of new chemicals to submit a PMN to EPA before they intend to begin manufacturing or importing. EPA has up to 90 days to evaluate whether the substance will

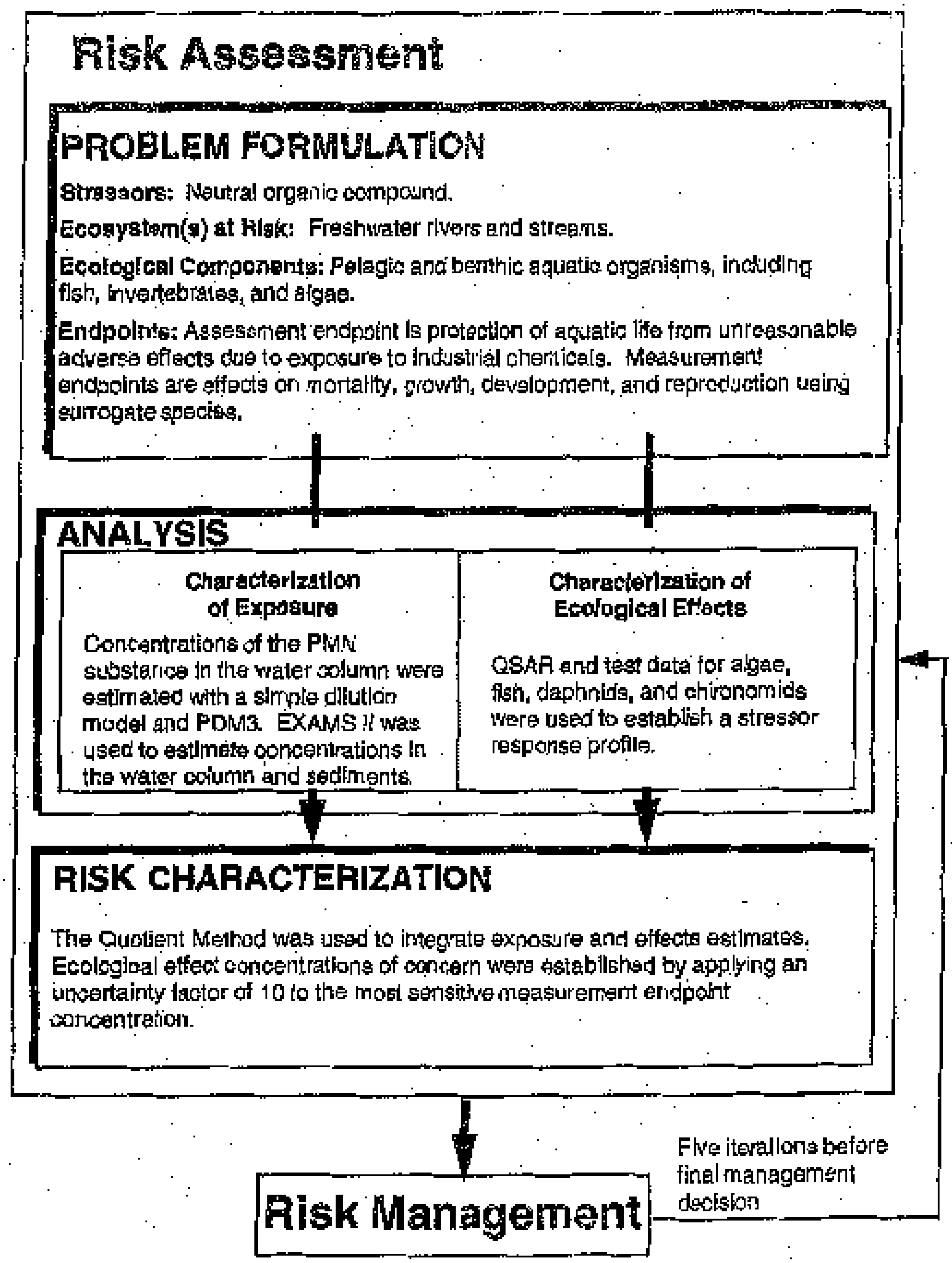

Figure 1-1. *Structure of assessment for effects of a PMN substance*

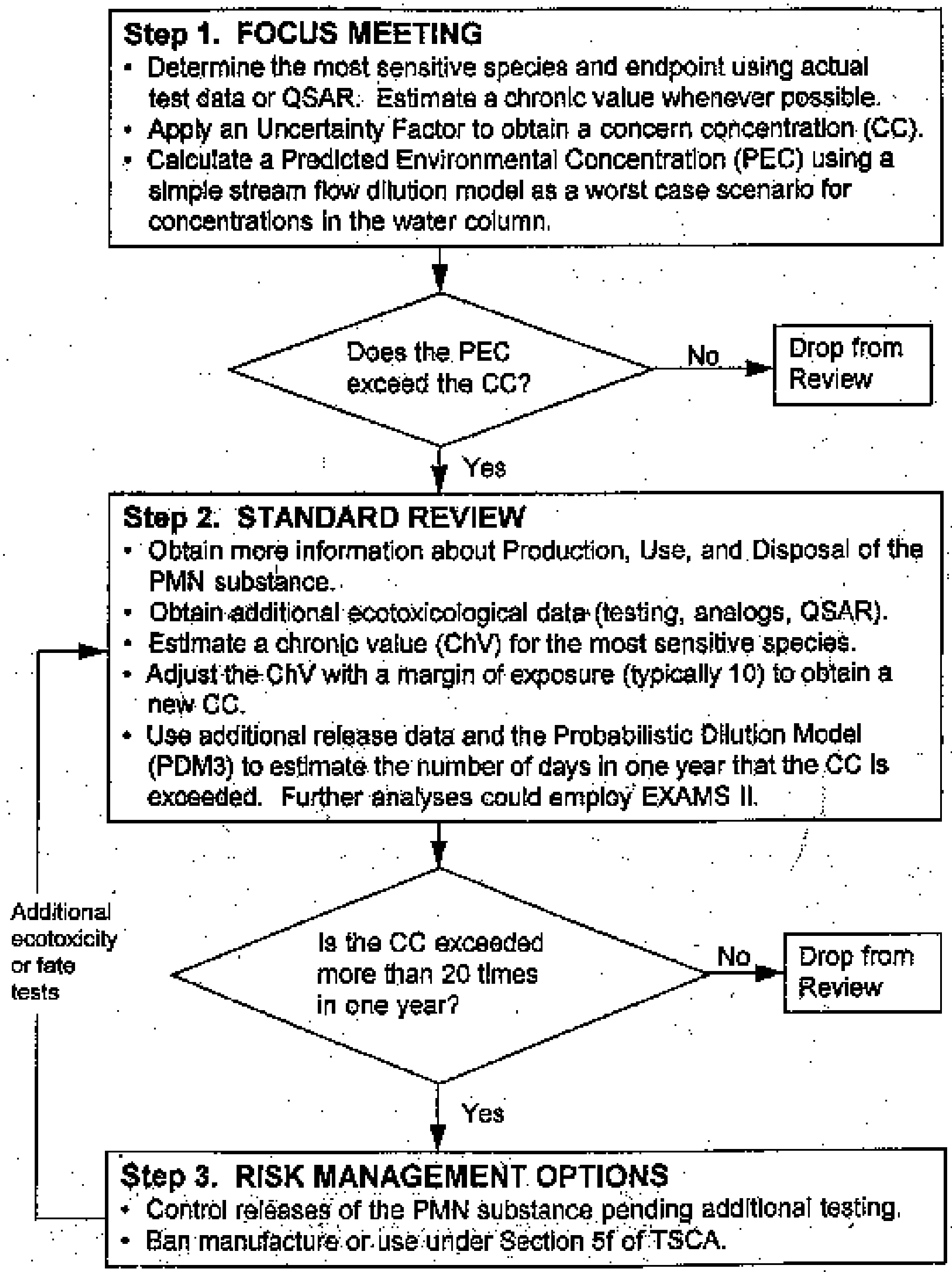

Figure 1-2. Flow chart and decision criteria for the ecological risk assessment of a PMN substance

present an unreasonable risk of injury to human health or the environment. With good cause, EPA can allow an extension of up to 180 days for the evaluation of the chemical.

In addition to the short review time allowed, there are three major problems associated with evaluating PMNs. The first is the confidential business information (CBI) protection afforded by TSCA. Under this clause, manufacturers and importers can designate many characteristics of the PMN substance, such as chemical name, structure, intended uses, and site of manufacture and use, as CBI. This information is not available to the public, and only personnel with TSCA CBI security clearance and members of Congress can access the information. There are strict safeguards against disclosure of the CBI (see text box on page 1-12). The second problem is that manufacturers and importers submit approximately 2,000 Section 5 notices to EPA annually. The third and perhaps the most important problem is that only the following information must be submitted: chemical identity; molecular structure; trade name; production volume, use, and amount for each use; by-products and impurities; human exposure estimates; disposal methods; and any test data that the submitter may have. The manufacturer does not have to initiate any ecological or human health testing prior to submitting a PMN. Only 4.8 percent of the PMNs reviewed to date contain ecological effects data, and most of those data consist of acute toxicity tests performed on fish (Nabholz, 1991; Nabholz et al., 1993a; Zeeman et al., 1993).

Confidential Business Information (CBI)

The CBI provisions of TSCA are intended to protect manufacturers and processors. Disclosure of chemical structures, uses, and even sites can provide competitors with proprietary information. However, CBI is available to the personnel involved with processing and evaluating Section 5 notices. This case study cannot provide certain information because of the CBI disclosure restrictions. Thus, this report does not reflect all available technical information, because certain details cannot be revealed to persons who are not cleared for CBI. For example, the technical assessors know the chemical name and structure of the PMN as well as the uses, sites, and releases, but such information cannot be revealed in this case study. Therefore, CBI does not hamper the ecological risk assessment process by EPA scientists who must be cleared initially for CBI before gaining access to such information. In addition, they must be certified on an annual basis to maintain their access to CBI. Once personnel move to positions that no longer require access to CBI, their clearance for access to such information is terminated.

1.3. CASE STUDY DESCRIPTION

This case study describes how OPPT evaluates the ecological risks of a PMN substance. The risk assessment begins with a worst-case analysis using a stream flow dilution model to estimate environmental concentrations. This is the typical approach taken by OPPT, and it results in very conservative estimates. Investigators initially use SARs to assess ecological effects, and the quotient method to integrate exposure and effects estimates.

Because the initial assessment identified a risk, additional analyses were performed using actual test data and PDM3. The second risk characterization indicated risks to pelagic and benthic aquatic life; therefore, investigators used the exposure analysis modeling system (EXAMS II) and generic site data to estimate concentrations in both the water column and sediments. Investigators estimated toxicity to benthic organisms using chronic test data for daphnids and assumed that the sediments would decrease toxicity by a factor of 10. The results of these analyses identified a risk.

The manufacturer then supplied OPPT with more precise data on the use and disposal of the PMN substance. Investigators input this new information into EXAMS II, and the results indicated little risk to benthic organisms at the identified sites. OPPT was ready to issue a consent order to restrict use of the PMN substance to the identified sites; however, the manufacturer chose to perform an actual test on benthic organisms using chironomids as the surrogate species. The results of the tests indicated moderate toxicity and little risk to benthic organisms at the identified sites. The final outcome was that EPA restricted the use of the PMN substance to the identified sites because there was uncertainty as to whether the concern level (1 μg/L) might be exceeded at sites not identified by the manufacturer.

1.3.1. Background Information and Objective

OPPT performs the following analyses in assessing the human and ecological risks of PMN substances. For a more detailed discussion of the process, see U.S. EPA (1986), Nabholz (1991), and Nabholz et al. (1993a).

1.3.1.1. Chemistry Report

The Industrial Chemical Branch of the Economics, Exposure and Technology Division (EETD) evaluates PMNs to ensure that: (1) the chemical name matches structure, (2) the chemical/physical properties are accurate, (3) the information about manufacturing and processing is accurate, and (4) the uses are consistent with the chemical.

1.3.1.2. Engineering Report

The Chemical Engineering Branch of EETD estimates worker exposure during the life cycle of the chemical (manufacturing, processing, use, and disposal) and estimates releases of the chemical to the environment.

1.3.1.3. Environmental Exposure Assessment

The Exposure Assessment Branch of EETD evaluates available fate, transport, and abiotic and biotic fate parameters. This is analogous to the exposure profile discussed in the Framework Report. The exposure assessment estimates the environmental concentrations likely to occur during the life cycle of the PMN substance. This includes an evaluation of potential exposure from releases to surface waters, landfills, and land spray, as well as nonoccupational exposures. Environmental concentrations can be site-specific or generic. PMN substances frequently are discharged to water; therefore, most exposure assessments address aquatic environments, chiefly rivers and streams.

1.3.1.4. Ecological Hazard Assessment

Also known as a toxicity assessment, the ecological hazard assessment is analogous to a stressor-response profile and is performed by the Environmental Effects Branch (EEB) of the Health and Environmental Review Division (HERD). The initial ecological hazard assessment evaluates the potential adverse ecological effects of a PMN substance and relies chiefly on SAR. For many classes of discrete organic chemicals (about 50 percent of which are neutral organic chemicals), quantitative structure-activity relationships (QSARs) are available that permit an estimation of acute and chronic effects to surrogate species such as fish, aquatic invertebrates, and algae (Auer et al., 1990; Clements, 1988; Nabholz et al., 1993a, b; Zeeman et al., 1993). HERD will review the results of submitted test data and, if the results are valid, incorporate them into the hazard assessment.

1.3.1.5. Ecological Risk Assessment

The Chemical Screening and Risk Assessment Division (CSRAD) conducts both human health and ecological risk assessments. Ecological risk assessments are conducted in a tiered fashion (figure 1-2). Initial hazard and exposure assessments are evaluated at a FOCUS meeting to ascertain whether a potential risk exists. If the FOCUS meeting does not identify a risk, the chemical may be dropped from further review. If a risk is identified, the PMN substance undergoes a more detailed

Table 1-1. Physical/Chemical Properties of PMN Substance

Property	Measured or Estimated Value
Chemical Class	Neutral Organic
Chemical Name	CBI
Chemical Structure	CBI
Physical State	Liquid
Molecular Weight	232
Log K_{ow}	6.7[a]
Log K_{oc}	6.56[b]
Water Solubility	0.051 mg/L (estimated)[c] 0.30 mg/L (measured)
Vapor Pressure	<0.001 Torr @ 20°C[d]

[a]Estimated using CLOGP program (Leo and Weininger, 1985).
[b]Estimated by a regression equation developed by Karickhoff et al. (1979). The average method error
for the log K_{oc} was 0.2 log K_{oc} units over a log K_{oc} range of 2 to 6.6.
[c]Estimated by a regression equation developed by Banerjee et al. (1980).
[d]Estimated by a regression equation cited in Grain (1982).

assessment called a standard review. Alternatively, additional information may be requested immediately following the FOCUS meeting. If a risk is still identified after all additional information has been submitted, then risk management options are considered. Possible risk management options are: (1) control options (such as no releases to water) pending further tests of the PMN substance, (2) issuance of a significant new use rule (SNUR), and (3) direct control under Section 5f (e.g., banning the manufacture or use of the PMN substance).

1.3.2. Problem Formulation

1.3.2.1. Stressor Characteristics

Table 1-1 lists the physical/chemical properties of the subject PMN substance. The manufacturer declared the chemical identity, structure, intended uses, and sites of use as CBI. This particular example evaluated only the parent compound, because investigators did not expect the PMN substance to degrade or be transformed into more toxic metabolites.

1.3.2.2. Ecosystem Potentially at Risk

The processing, use, and disposal sites are adjacent to rivers and streams. Investigators also expected the PMN substance to be discharged to such rivers and streams. Thus, pelagic and benthic aquatic populations and communities may be at risk.

1.3.2.3. Ecological Effects

The PMN substance belongs to a class of chemicals known as neutral organic compounds. These chemicals are nonelectrolyte and nonreactive and exert toxicity through a narcotic or nonspecific mode of action (Auer et al., 1990; Lipnick, 1985; Veith and Broderius, 1990). Neutral organic compounds can exert both acute and chronic effects. The toxicity of neutral organic compounds has been correlated with molecular weight and the logarithm of the octanol-water partition coefficient (K_{ow}). Experimental data have shown that neutral organics with a log K_{ow} of 5.0 or more do not exert pronounced acute effects (toxic effects such as mortality or immobilization within 4 days). This is mainly due to the low water solubility of such compounds, which results in decreased bioavailability to aquatic organisms. Because of the decreased bioavailability, exposure durations of 4 days or less are insufficient to elicit marked acute effects (e.g., as measured by a 96-hour LC_{50}[1] test). Because of the high K_{ow} of this PMN substance, investigators expected only chronic effects to occur at or below the chemical's aqueous solubility limit.

OPPT typically assesses ecological effects for three trophic levels: primary producers (algae), primary consumers (aquatic invertebrates), and forage/predator fish. Investigators use the most sensitive species and toxicological effect for the initial risk assessment. Unless only chronic effects are expected, such as the PMN substance in this case study, OPPT usually assesses both acute and chronic effects. The ecological effects characterization is based on effects on mortality, growth and development, and reproduction. The rationale and approach used to assess these effects are presented under Measurement Endpoints.

1.3.2.4. Assessment Endpoints

TSCA was intended to prevent unreasonable risks to health and the environment as a result of the manufacture, processing, use, and disposal of industrial chemicals. The assessment endpoint (Suter, 1990) used in this case study is the protection of aquatic organisms (algae, aquatic invertebrates, and fish). The investigators assumed that any effects from the PMN substance would be exhibited at least up to the population level of organization.

1.3.2.5. Measurement Endpoints

Investigators used the following measurement endpoints (Suter, 1990) to assess the risks to the assessment endpoint:

- mortality;
- growth and development; and
- reproduction.

Clements (1983) and U.S. EPA (1984) present the rationale for selecting these endpoints. To summarize, documented evidence indicates that xenobiotics can adversely affect these endpoints both directly and indirectly. Since populations are governed by mortality, growth and development, and reproduction, investigators presumed that adverse effects to these measurement endpoints would manifest themselves at least up to the population level of ecological organization. Thus, there is a logical connection between the assessment endpoint (i.e., the protection of aquatic life, at least up to the population level) and the measurement endpoints.

OPPT uses a tiered approach when testing the toxicity of a given industrial chemical (U.S. EPA, 1983; Smrchek et al., 1993; Zeeman et al., 1993). The first tier consists of relatively inexpensive short-term tests that measure effects chiefly on mortality to fish and aquatic invertebrates and population growth for green algae (the three trophic levels discussed under Ecological Effects). The first tier or "base set" consists of a 96-hour fish acute test, a 48-hour daphnid test, and a 96-hour algal test. Because the algal test represents exposure across about eight generations of algal cells,

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[1]The LC_{50} is the median lethal concentration.

OPPT considers the algal test to be representative of chronic toxicity to algal populations. Additional tiers consist of chronic tests, such as the fish early life stage toxicity test that measures effects on mortality and growth and development, and the daphnid chronic test that measures effects on survival and reproduction. Investigators must ascertain a risk before proceeding to these additional tests.

1.3.2.6. Conceptual Model

Based on experience with neutral organic compounds and available QSARs, the high log K_{ow} for the PMN substance indicated a risk of chronic toxicity to benthic and pelagic aquatic organisms. Principal concerns were for effects on mortality, growth and development, and reproduction. Investigators presumed that these effects would be manifested at least up to the population level of organization (Clements, 1983).

A preliminary exposure profile was developed through the use of simple stream flow models. To characterize ecological effects, QSARs were used to develop an initial stressor-response profile (Clements, 1988). The QSARs established which trophic level (i.e., algae, fish, aquatic invertebrates) would be the most sensitive, and were developed from actual tests of neutral organic compounds using surrogate species (U.S. EPA, 1982) that represented aquatic organisms in rivers and streams.

Assessment factors (U.S. EPA, 1984; Nabholz, 1991; Nabholz et al., 1993a) were used to address uncertainties in extrapolating from laboratory to field effects. Investigators used a quotient method of ecological risk characterization to assess risk (Barnthouse et al., 1986; Nabholz, 1991; Rodier and Mauriello, 1993). If the results of the risk characterization predicted an unreasonable risk, investigators planned to perform a more in-depth analysis including fate and transport modeling and ecological effects testing in accordance with EEB ecological effect test guidelines (U.S. EPA, 1985). The PDM3 and EXAMS II models would further characterize and refine exposure, and additional ecological effects testing of the PMN substance would be based on the criteria established by OPPT (U.S. EPA, 1983). Investigators would continue to use the quotient method to characterize risks.

Comments on Problem Formulation

Strengths of the case study include:

- *The process is scientific and judged to be adequate.*
- *The case study is a good example of the PMN process.*

Limitations include:

- *Much of the information is confidential and is unavailable to the reviewers.*
- *The problem formulation section should present more detail on potential ecological effects.*
- *The PMN process appears to consider chemicals singly and not as part of a complex mixture in the environment. Other chemicals might interact with the chemical of interest, thereby changing exposure and/or toxicity.*
- *There should be some discussion as to the potential for transformation products and what might be done if they were known to be produced.*

General reviewer comments:

- *This case study addresses all components of a risk assessment listed in the EPA's Framework Report.*
- *Future PMN assessments should include fairly realistic, yet simple, bioaccumulation models.*

Comments on Problem Formulation (continued)

Author's comments:

- *Using a general assessment endpoint, such as the protection of aquatic organisms, helps to communicate the significance of risks determined with measurement endpoints. Risk managers might not be familiar with the surrogate species used in PMN testing or the significance of the test results (e.g., EC_{50}, MATC).*
- *Given the volume of PMNs received annually, the approach of using conservative methods initially and then proceeding to more detailed assessments, as necessary, is the only practical approach.*
- *Generic assessments cannot identify specific biota at risk. This often is considered a shortcoming; however, given the conservative exposure estimates provided by the stream flow models, the lack of information about biota at specific sites, and the use of assessment factors for projecting ecological effects, it is not unreasonable to assume that the risk assessment will protect a wide array of aquatic organisms.*
- *TSCA gives no legislative authority to regulate mixtures of chemicals. TSCA is written to address each chemical individually.*
- *OPPT always considers potential transformation products during assessments. If a persistent and/or more toxic transformation product could be formed from a PMN substance, OPPT would assess the product in the same way as the parent compound was assessed. In this case, no transformation products of concern were identified.*
- *PMN assessments do include bioaccumulation models when they are needed. Fish ingestion models by humans is a standard model run for all PMN substances. Fish ingestion by predators is assessed if a potential concern has a likely probability of occurring. In the early stages of this case, the assessor knew that food chain transport could be a problem. Late in the assessment, the company submitted fish bioconcentration data for a close analog, which showed that the measured fish bioconcentration factor of the PMN substance would be much lower than predicted. Therefore, exposure to human populations and predators through fish ingestion was not evaluated further.*

1.3.3. Analysis, Risk Characterization, and Risk Management—1st Iteration

1.3.3.1. Analysis: Characterization of Exposure

Because the use of the PMN substance is CBI, only the terms Manufacturing, Processing, Use, and Disposal are used to describe the life cycle of the compound. The sites of manufacture, use, and disposal are CBI, and this draft considers the actual releases that were used to calculate concentrations of the PMN substance in receiving rivers and streams as CBI.

1.3.3.1.1. Stressor Characterization

The compound has low water solubility and is not expected to volatilize from water because of the low vapor pressure. Photodegradation is negligible, and the compound is expected to sorb strongly to sediments. The half-life for aerobic degradation could be weeks; anaerobic degradation could require months or longer.

Table 1-2. PECs for PMN Substance (μg/L)

Process	Mean Flow		Low Flow	
	10%[a]	50%	10%	50%
Manufacture	0.0	0.0	0.0	0.0
Use	9.0	0.5	68.0	4.0
Disposal	52.3	0.7	90.2	6.1

[a]Percentage of streams having flows equal to or less than the value used to calculate the PECs.

1.3.3.1.2. Exposure Analysis

In the first iteration, investigators used a simple stream flow dilution model to calculate predicted environmental concentrations (PECs). The calculation was based on the following algorithm:

Concentration = Releases (kg/day) / Stream flow (millions of liters/day)

The PEC calculations use both mean and low flow rates. In addition, the initial OPPT exposure analysis typically ranks stream flow rates and uses the 10 percent and 50 percent flow rates. The measured solubility limit of 0.3 mg/L was used.

Investigators determined that there would be no significant releases during the manufacture of this PMN substance. The most significant routes of exposure would result from the use and disposal of the chemical. Effluents containing the PMN substance would first be treated in publicly owned treatment works (POTW), which are wastewater treatment plants that include primary and biological treatment of the incoming waste stream. POTWs normally are located off-site or between the processing plant and the receiving river. To assess the extent of removal of the PMN substance by POTWs, investigators used data from laboratory-scale wastewater treatment experiments and the output from mathematical wastewater treatment simulations. The results indicated that removal would be due largely to adsorption to sludge; however, the analysis assumed approximately 10 percent of the PMN substance released from treatment was in the effluent sorbed to solids. This assumption was based on typical solids removal for secondary wastewater treatment systems.

This study did not consider the fate and ecological effects of the PMN substance in sludge.

1.3.3.1.3. Exposure Profile

Table 1-2 lists the PECs for the PMN substance during manufacture, use, and disposal.

1.3.3.2. Analysis: Characterization of Ecological Effects

OPPT initially used QSAR to estimate the ecological effects of the PMN substance. The manufacturer contacted EPA prior to submitting the PMN and was briefed on concerns about chronic effects. As a result, the manufacturer submitted a fish acute test and a fish early life stage test.

1.3.3.2.1. Stressor-Response Profile

Table 1-3. PMN Substance Stressor-Response Profile

QSAR Estimated Toxicity[a]		
Endpoint	**Effect Concentration**	**Reference**
Fish 96-hr LC_{50}	No effect at saturation	Veith et al. (1983)
Daphnid 48-hr LC_{50}	No effect at saturation	Hermens et al. (1984)
Green Algae 96-hr EC_{50}[b]	No effect at saturation	Appendix A
Fish ChV[c]	0.002 mg/L	Appendix A
Daphnid ChV	0.004 mg/L	Hermens et al. (1984)
Algal ChV	No effect at saturation	Appendix A
Actual Measured Toxicity		
Fathead Minnow (*Pimephales promelas*) 96-hr Acute Test	No effect at saturation	U.S. EPA (1993)
P. promelas Early Life Stage Test, 31-day ChV (growth, mean wet weight)	0.013 mg/L	U.S. EPA (1993)
P. promelas Early Life Stage Test, 31-day ChV (survival, growth [length])	0.061 mg/L	U.S. EPA (1993)

[a]Based on molecular weight and log K_{ow}.
[b]Median effect concentration.
[c]The ChV is the geometric mean of the highest concentration for which no effects were observed and lowest concentration for which toxic effects were observed. The ChV is essentially the geometric mean of the maximum acceptable toxicant concentration (MATC).

Table 1-3 summarizes the QSAR-derived effect concentrations and the results of the fish acute and fish early life stage tests.

1.3.3.3. Risk Characterization

Five risk characterizations were performed in this case study. Table 1-4 provides a brief summary of the assumptions, estimations, and types of uncertainty for each of the five iterations.

1.3.3.3.1. Risk Estimation (Integration and Uncertainty Analysis)

Investigators used the quotient method to estimate ecological risks. A quotient of 1 or greater indicates a risk. The algorithm is given below:

$$\text{Risk Quotient} = \text{PEC/CC}$$

Normally, OPPT calculates the concern concentration (CC) by identifying the most sensitive species and effect from the stressor-response profile and applying an assessment factor. In this case,

Table 1-4. Summary of Five Risk Characterization Iterations

Iteration	Estimates/Assumptions	Uncertainty
1	Fish are the most sensitive species. Chronic effects at 1 μg/L. PMN substance mixes instantaneously in water. No losses.	Worst-case analysis.
2	Actual test data for daphnids still indicate a ChV of 1 μg/L. Determine how often this concentration is exceeded using PDM3.	Worst-case analysis. Other species may be more sensitive.
3	Estimate risk to benthic organisms using daphnid ChV and mitigation by organic matter. EXAMS II used to estimate concentrations.	Generic production sites. Actual data for benthic organisms not available.
4	Site-specific data obtained on use and disposal. EXAMS II rerun with new data.	Estimated toxicity for benthic invertebrates.
5	Actual test data for benthic organisms obtained.	Best estimates for identified sites. May not hold for other sites or uses.

investigators used the measured chronic value (ChV) of 0.013 mg/L for the fathead minnow rather than the estimated ChV of 0.004 mg/L for the daphnids (table 1-3). To account for the uncertainty between chronic effects noted in the laboratory and those that might occur in the field, an assessment factor of 10 was used (see text box on page 1-22). The ChV was divided by the assessment factor to yield a CC of 0.0013 mg/L, which was rounded off to 0.001 mg/L or 1 μg/L.

In estimating risk, the CC of 1 μg/L was compared to the PECs (table 1-2). As can be seen, the CC was exceeded at both low and mean flow for 10 percent of the streams, and at low flow for 50 percent of the streams. A risk was inferred based on mean flow.

It should be noted that the initial risk assessment evaluates risks to aquatic species in the water column only.

1.3.3.4. Risk Management

Because the results of the initial risk characterization identified a potential unreasonable risk, investigators requested a chronic daphnid test to complete the chronic tier tests. EPA also informed the submitter that a benthic test with contaminated sediments could be required if there was a potential unreasonable risk to sediment-dwelling organisms. The concern for benthic organisms was based on the high K_{ow}, low vapor pressure, and low water solubility, which indicate that the PMN substance was likely to partition to the sediments of rivers and streams, resulting in exposures of benthic organisms. EPA also requested a coupled units test (40 CFR 796.3300) to simulate the effectiveness of a POTW in removing the PMN substance.

Uncertainty Assessment Factors

OPPT uses assessment factors to attempt to address three types of uncertainty:

- *Uncertainty regarding differences in species sensitivity to toxicants.*
- *Uncertainty regarding the differences between concentrations eliciting acute effects and those causing chronic effects.*
- *Uncertainty regarding comparisons of laboratory studies to field conditions.*

Assessment factors range from 1 to 1,000. The particular assessment factor used for a chemical will vary inversely with the amount and type of data available. Examples are shown below. A complete discussion can be found in U.S. EPA (1984).

Examples of Assessment Factors

Available Data	*1,000*
Acute toxicity QSAR or test data for one species	*100*
QSAR or test data for fish, algae, and aquatic invertebrates	*10*
QSAR or chronic toxicity data for fish or aquatic invertebrates	*1*
Actual field study	
Assessment Factor	

1.3.4. Analysis, Risk Characterization, and Risk Management—2nd Iteration

1.3.4.1. Characterization of Ecological Effects

A daphnid chronic toxicity test was conducted and found to be acceptable (i.e., it followed OPPT guidelines and good laboratory practices). The ChV for survival, growth, and reproduction was 0.007 mg/L.

1.3.4.2. Characterization of Exposure

The coupled units test is a measure of the ultimate biodegradation of the PMN substance under conditions that simulate treatment in activated sludge. The POTW simulation conducted by the manufacturer indicated that a POTW would remove from 95 percent to 99 percent of the PMN substance.

1.3.4.3. Risk Characterization

Investigators used PDM3 (U.S. EPA, 1988) to estimate the number of days out of 1 year that the CC will be exceeded. Like the simple stream flow model, PDM3 assumes that the chemical will mix instantaneously with water and no losses will occur through any physical, chemical, or biological transformations. Flow rates were obtained from the U.S. Geological Survey.

Investigators continued to use the CC of 1 μg/L, since the daphnid ChV of 0.007 mg/L divided by the assessment factor of 10 rounds off to 0.001 mg/L or 1 μg/L.

Table 1-5. PDM3 Analysis[a]

Process	Exceedance (days/year)
Manufacture	0
Use	20
Disposal	39

[a]Releases to water considered CBI. PMN substance was expected to be released 350 days/year, and a 95 percent removal from POTW was assumed.

Table 1-5 presents the results of PDM3.

1.3.4.3.1. Interpretation of Ecological Significance

As a matter of policy, OPPT infers an unreasonable risk to aquatic organisms if a CC for chronic effects exceeds 20 days or more. The 20-day criterion is derived from partial life cycle tests (daphnid chronic and fish early life stage tests) that typically range from 21 to 28 days in duration. OPPT infers a reasonable risk if the CC is exceeded less than 20 days. It is important to remember that the PDM3 model estimates only the total number of days out of 1 year that the CC is exceeded. The days are not necessarily consecutive, and thus the 20-day criterion is a conservative one. This iteration showed an unreasonable risk to aquatic organisms from the PMN substance because the CC was exceeded 20 days for use and 39 days for disposal (table 1-5).

1.3.4.4. Risk Management

EPA notified the company that a potentially unreasonable risk to aquatic organisms still existed. A meeting was held to discuss possible benthic toxicity tests and to clarify unanswered questions regarding releases of the PMN substance through use and disposal. It also was decided to evaluate exposure further through the use of EXAMS II (Burns, 1989).

1.3.5. Analysis, Risk Characterization, and Risk Management—3rd Iteration

1.3.5.1. Characterization of Ecological Effects

Currently, there are no SARs for neutral organics and aquatic benthic organisms; however, SARs do exist for neutral organics with earthworms in artificial soil. To estimate the ecological effects of the PMN substance to aquatic benthic organisms, predictions from the fish 14-day LC_{50} QSAR (Konemann, 1981) were compared with the earthworm 14-day LC_{50} QSAR. The earthworm 14-day LC_{50} was about 10 times higher than the fish 14-day LC_{50}. Investigators assumed that the organic matter (i.e., ground peat) in the artificial soil mitigates the toxicity of neutral organic chemicals by about 10 times.

Investigators further expected that the organic matter in natural sediments would mitigate the toxicity of the PMN substance by at least a factor of 10, because natural organic matter in natural sediments should be more efficient at binding neutral organic chemicals than freshly ground peat in artificial soil. That is, sediment organic matter is likely to have a larger surface area-to-volume ratio than ground peat and, therefore, have more sites to bind hydrophobic compounds. Proceeding on the above assumption, the effective concentrations in the toxicity profile for water column were multiplied by 10 to produce the stressor-response profile for benthic organisms (table 1-6). This scenario used the best data available at the time for neutral organic compounds, and the PMN submitter accepted the rationale for mitigation.

Table 1-6. (Estimated) Stressor-Response Profile for Benthic Organisms

Organism	Endpoint	Effect Level (mg/kg dry weight)
Invertebrate	14-day LC_{50}	0.3
Invertebrate	21-day ChV	0.10
Vertebrate	31-day ChV	0.3 to 1.0

1.3.5.2. Characterization of Exposure

A preliminary EXAMS II analysis at the worst site indicated concentrations ranging from 11.2 to 21.8 mg/kg dry weight of sediment after 1 year of releases of the PMN substance. Appendix B presents the critical input parameters for EXAMS II and an example of the output.

1.3.5.3. Risk Characterization: Risk Estimation and Uncertainty Analysis

The most sensitive endpoint was the invertebrate 21-day ChV of 0.1 mg/kg. An assessment factor of 10 was applied to derive a CC of 0.01 mg/kg or 10 µg/kg. The quotient method was used. As can be seen from the initial EXAMS II analysis, the exposure concentrations exceeded the CC by factors of 1,000 to 2,000.

1.3.5.4. Risk Management

The manufacturer initiated an extensive site-specific evaluation of the releases of the PMN substance during uses and disposal, and submitted new exposure information to OPPT for evaluation. The report is CBI.

1.3.6. Analysis, Risk Characterization, and Risk Management—4th Iteration

1.3.6.1. Characterization of Exposure

OPPT used the additional information to conduct another EXAMS II analysis. Table 1-7 summarizes the results for three representative sites.

Table 1-7. EXAMS II Analysis

Site	Water Column (µg/L)	Sediments (mg/kg)
1	0.004	0.019
2	0.001	0.014
3	0.008	0.038

Table 1-8. Stressor-Response Profile for *Chironomus tentans*

Endpoint	Effect Level (mg/kg dry weight sediment)
14-day ChV	32
21-day EC_{50} emergence	23
25-day EC_{50} emergence	25
28-day EC_{50} emergence	24
28-day LC_{50} survival	22
ChV survival	23
ChV emergence	23

1.3.6.2. Risk Characterization

There was not enough of a risk to benthic organisms to warrant a ban pending a testing decision by OPPT.

1.3.6.3. Risk Management

A decision was made to offer the company a consent order to allow manufacturing but require a benthic/sediment toxicity test to confirm the toxicity profile and thus the risk assessment. Prior to offering the consent order, the company volunteered to test with a benthic organism using contaminated sediment. The submitter and OPPT agreed to a 28-day chironomid toxicity test.

1.3.7. Analysis, Risk Characterization, and Risk Management—5th Iteration

1.3.7.1. Characterization of Exposure

Table 1-8 presents the results of the chironomid toxicity test.

1.3.7.2. Risk Characterization—Risk Estimation

A CC of 2.0 mg/L was set for the benthic community based on the most sensitive effect, a ChV of 23 mg/kg for survival and emergence. The CC was 50 times higher than the highest PEC for sediments, and the ChV was 600 times higher. Thus, there did not appear to be an unreasonable risk to benthic organisms as a result of the use and disposal of the PMN substance over 1 year.

As can be seen from table 1-7, concentrations of the PMN substance were three orders of magnitude lower than the concern level of 1 μg/L for water column organisms at the specific sites of use and disposal.

1.3.7.2.1. Uncertainty

In this case study, the three main types of uncertainty with regard to ecological effects are variations in species-to-species sensitivity, uncertainty regarding acute versus chronic effects, and uncertainty regarding extrapolating laboratory-observed effects to those that might occur in the natural environment. U.S. EPA (1984) developed assessment factors specifically for establishing concentrations of concern for PMN substances. Use of these factors is not intended to establish a "safe" level for a particular substance, but rather to identify a concentration which, if equaled or exceeded, could result in some adverse ecological effects. Such a finding provides the rationale for requesting either actual testing of the PMN substance or more specific information about fate and exposure. Naturally, there are other types of uncertainty, such as the effects of the PMN substance on adult rather than juvenile fish. Such types of uncertainty are research issues.

In the case of the exposure profile, an important aspect of uncertainty has to do with the actual duration of exposure. The PDM3 model predicts only the number of days out of 1 year that the CC will be exceeded (table 1-4). These days are not necessarily consecutive days. Thus, only flow rates could be used to account for seasonal variation. The presence or absence of critical life stages of aquatic organisms cannot be accounted for with this type of analysis. In addition, the generic nature of the assessment precludes identification of specific biota.

1.3.7.2.2. Risk Description—Ecological Risk Summary

This case study demonstrates the validity of QSAR in establishing toxicity profiles for water quality organisms (fish, invertebrates, and algae). In this case, the chemical structure indicated that the PMN substance was closely analogous to chemicals known to behave like neutral organic compounds. The high K_{ow} indicated that the compound would not be acutely toxic, and this was confirmed by an actual test with a surrogate fish species. Actual chronic toxicity testing confirmed the QSAR-predicted chronic toxicity (within an order of magnitude). EPA's experience with other high-K_{ow} compounds such as hexachlorobenzene and chloroparaffins further confirms the chronically toxic nature of such compounds. The predictions for chironomid toxicity did not agree with the actual test data. QSARs have not been developed for benthic organisms simply because not enough test data are available to permit such analyses.

The use of QSAR is not limited to neutral organic compounds. Currently, there are QSARs available for compounds that show more specific modes of toxicity or excess toxicity over the neutral organics. Examples include acrylates, methacrylates, aldehydes, anilines, benzotriazoles, esters, phenols, and epoxides (Auer et al., 1990; Clements, 1988).

Because the CCs were exceeded enough times out of 1 year, the PDM3 model indicated a risk to aquatic organisms. When actual sites were analyzed using EXAMS II, no unreasonable risks were identified.

1.3.7.2.3. Ecological Significance

There appears to be no unreasonable risks to pelagic and benthic organisms at the identified use sites. The potential risk posed by the PMN substance bioaccumulating through the aquatic food web was thought not to be significant.

1.3.7.2.4. Spatial and Temporal Patterns of the Effects

CBI restrictions preclude revealing the uses and specific sites for the PMN substance. However, the technical assessors identified important river systems that could be affected by this PMN substance. Thus, if there was a risk, the effects are not likely to be localized.

1.3.7.2.5. Recovery Potential

The PMN substance is a neutral hydrophobic chemical. This mode of toxicity is akin to a simple narcosis type of action (Auer et al., 1990; Veith and Broderius, 1990) that is reversible if exposure to the toxicant is terminated before lethality or death occurs.

The recovery potential was not evaluated. Short-term pulsed exposure is not likely to cause adverse effects. However, continued exposure is likely to cause some impact to benthic organisms, but not enough of an impact to regulate.

1.3.8. Risk Management—Final Decision

The risk managers agreed that the PMN substance posed no unreasonable risks to pelagic aquatic organisms at the specific sites of use and disposal. However, there could be risks at other sites through the use and disposal of the PMN substance. Therefore, the final disposition was a SNUR including a restriction against releasing concentrations higher than 1 μg/L (the concern level for the PMN substance). The manufacturer must submit a significant new use notice if it wants to use the PMN substance at sites other than the ones identified in its submission.

Comments on Characterization of Exposure

Strengths of the case study include:

- *The case study used a well-known model for estimating exposure in aquatic environments. The case study illustrates how such models can be employed.*
- *The study relied on a pilot treatment study to estimate removal of the chemical in a POTW (i.e., with the sludge).*
- *The study provides a good example of the PMN review process.*

Limitations include:

- *The choice of K_{ow} or K_{oc} value is a critical aspect of the study that should be discussed. Also, the case study should include a discussion of the sensitivity of the results to selection of K_{ow} values.*
- *Chemical properties indicated a tendency to bind suspended particles, yet there was no exposure pathway involving chemical → sediment → suspended particle → feeding or bioconcentration.*

Comments on Characterization of Exposure (continued)

- *The exposure analysis should have considered the fate of the sludge from the POTW. Such sludge is often applied to agricultural or forest land.*
- *More detail about the discharges should be given, even if it is something like "one large" or "several small."*
- *For chemical products that are mixtures, there may be a large number of chemicals present, and this may contribute to variability in estimates as well as measurements. The mixture can result in exposure conditions in the environment different from those for the original material.*

Author's comments:

- *The simple stream flow model offers a conservative estimate of exposure by assuming instantaneous mixing and dispersion of the chemical. The model does not take into account any losses due to factors such as volatilization, partitioning, or chemical or biological degradation after release. Because of the paucity of data and information about exposure, the use of conservative models is justified.*
- *The PDM3 model is an improvement over the simple stream flow models in that the temporal nature of exposure can be evaluated. Thus, a risk manager can be advised as to how often a particular concentration is likely to be exceeded.*
- *The above two models estimate chemical concentrations in the water column only. As demonstrated in the study, more in-depth models such as EXAMS II can be used to estimate chemical concentrations both in the water column and sediments when sufficient data are available.*

Comments on Characterization of Ecological Effects

Strengths of the case study include:

- *The case study illustrates the iterative approach associated with the evaluation of a PMN chemical.*

Limitations include:

- *Estimating a concern level for sediment organisms based on earthworm data is not appropriate because earthworms exchange gases with air and sediment organisms exchange gases with water. The statement that sediment organic carbon will mitigate toxicity 10 times more than soil or peat carbon requires additional support.*

Comments on Characterization of Ecological Effects (continued)

- *The authors should give a good rationale for their approach to estimating sediment toxicity and tell why it is better than the equilibrium partitioning method other offices in EPA are using, or they should use the equilibrium partitioning method.*
- *The case study should not state that it demonstrates the validity of QSAR in establishing toxicity profiles. The estimated 21-day chronic value of 0.100 was 230-fold lower than the test results (tables 1-6 and 1-8). A single case study would not be sufficient to demonstrate the validity of using QSAR to establish toxicity profiles, no matter what might have been shown.*

Author's comments:

- *The use of SAR is commonplace within OPPT because TSCA does not require the completion of test data prior to the submission of a PMN. The track record with SAR is extremely good (Nabholz et al., 1993b) and has resulted in dropping low- risk chemicals from review and regulating high-risk chemicals without measured data on the chemical. Provided sound expert judgment is employed, SAR can identify whether acute or chronic tests (or both) are needed.*
- *The use of surrogate species at different trophic levels (e.g., fish, daphnids, algae, benthic organisms) permits one to evaluate which organisms are most sensitive to a given xenobiotic. While many argue that the commonly used surrogates may not be as sensitive as those in the wild, both industry and government agree that it is the most practical way to evaluate the ecological effects of chemicals. Because many industrial chemicals are used in a wide array of industries as well as consumer products, identifying specific biota (at the species level) is often impossible.*
- *The cost of larger scale studies such as laboratory microcosms and field mesocosms has precluded their use to assess the ecological effects of new chemicals. However, OPPT is initiating field mesocosm studies at the ERL-Duluth to evaluate how well laboratory tests predict effects in the field.*

Comments on Characterization of Ecological Effects (continued)

- *Comparing a fish 14-day LC_{50} with an earthworm 14-day LC_{50} value in an artificial soil was the only available way at the time to estimate the effect that organic matter would have on the bioavailability of an organic chemical in sediments. It was known that (1) earthworms interacted intimately with soil pore water, (2) the toxicity of organic chemicals in soil toward earthworms could be predicted by relating molar concentration in soil pore water to the chemical's K_{ow} (van Gestel and Ma, 1990), (3) K_{ow} was highly correlated with K_{oc}, and (4) the amount of organic matter in sediments strongly influenced the amount of organic chemical that could be absorbed by sediments. It was a simple and valid extrapolation to use earthworms as a surrogate for benthic organisms. In addition, when the OPPT assessment team conferred with the submitter's assessors, the submitter accepted OPPT's best estimate given the level of knowledge and available data that existed at the time.*

- *Sediment organic matter was expected to be more efficient than the ground peat used in the artificial soil of the earthworm toxicity test because sediment organic matter is generally more finely divided due to more processing by invertebrates and partial degradation by microbes. Sediment organic matter was expected to have a much greater surface area to volume ratio than the peat and, therefore, a much greater absorptive area to reduce the bioavailability of organic chemicals with high K_{ow} values (i.e., >4.2).*

Comments on Risk Characterization

Strengths of the case study include:

- *The risk characterization appeared to be adequate for a management decision.*

- *The case study illustrates the PMN risk assessment process.*

Limitations include:

- *The summary table of the major assumptions and estimates used at various stages of the process (table 1-4) should have included some information on the magnitude of uncertainty associated with each of these estimates or assumptions.*

- *The risk assessment methods employed do not distinguish risks to individuals from risks to populations. In some cases "individuals" are the organizational level of interest, while in other cases it is the "population."*

Comments on Risk Characterization (continued)

- *It was pointed out that assessment factors were developed in 1984 and continued to evolve along with the PMN process. The case study should include the method used to derive the assessment factors and a brief statement of the history of these factors.*
- *The case study should clarify the difference between "uncertainty" in the statistical sense and "uncertainty" as it is addressed by using "assessment factors."*
- *Assigning an assessment factor of "1" to field toxicity data is not appropriate because field data are site-specific, and the data may not be directly transferable to other sites where the chemical might be used or released.*
- *The available information suggested that risks to benthic organisms were probably more important than those to pelagic organisms. Yet, the process was carried out in a specific manner that emphasized the studies on pelagic organisms first. It was pointed out that this was policy.*
- *If disposal options were considered along with the risk assessment, then various options could have been considered early in the process. Such mitigation could be included as an iteration.*
- *If "acceptable" concentrations were first identified, then it would be possible to estimate acceptable loadings.*

General reviewer comments:

- *The case study description of interactions between risk assessors and risk managers led the reviewers to discuss the following:*
  - *Who is the risk manager? It appears to be a manager at EPA, but the manager at the company also can manage risk by deciding not to test further and abandoning the chemical, or he could deal with potential exposure by treating the waste stream or making process changes.*
  - *It might be useful to develop a framework for risk managers similar to that for risk assessors. Both frameworks should contain sections on interaction with the other and on mitigation.*
- *The example chemical does not indicate how well the process works for other types of chemicals. Narcoleptic chemicals are the easiest chemicals to model for toxicity. Reactive toxicants often cannot be modeled simply, if at all, and they are usually more toxic or hazardous.*

Comments on Risk Characterization (continued)

- *Reviewers discussed the use of "probabilistic" risk assessment. It was noted that this is the direction in which EPA is going. A question was raised regarding whether these quantitative methods would be understandable to the risk manager. Some experience indicates that they would.*

Author's comments:

- *The Quotient Method is the most common ecological risk assessment method used in OPPT for new and existing chemicals. It also is used by the Office of Pesticide Programs. The Quotient Method is easy to use, is mutually accepted by industry and EPA, and is amenable to the ecological effects and exposure data available to OPPT under TSCA.*

- *One disadvantage of this method is the uncertainty about the degree of risk when quotients approach, but do not equal, 1. Also, it is difficult to quantify risks to assessment endpoints when most ecological risk assessments under TSCA are generic. While the risks to measurement endpoints can be quantified, extrapolating such risks to the population or community level is impossible unless simulation models are employed. OPPT is evaluating developmental versions of population and ecosystem models for use with existing chemicals; however, due to the volume of PMNs, their use is not practical at this time. This is particularly true for ecosystem models that require mainframe or high-speed/high-memory computers. Thus, only qualitative inferences can be made between measurement and assessment endpoints.*

- *Since 1979, OPPT has assessed the environmental toxicity of over 24,000 chemicals submitted under Section 5 of TSCA. Although only 4.8 percent of those chemicals had any environmental toxicity information submitted with them, OPPT has been able to use chemical structure and commonly measured physical/chemical properties to model the aquatic toxicity of many classes of reactive toxicants, including 64 classes of organic chemicals that have some type of specific toxicity in addition to narcosis.*

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APPENDIX A

QSARS BETWEEN NEUTRAL ORGANIC CHEMICALS AND FISH AND GREEN ALGAL TOXICITY

QSARS BETWEEN NEUTRAL ORGANIC CHEMICALS AND FISH CHRONIC VALUES

(Broderius and Russom, 1989)

- $\text{Log NOEC (mol/L)} = -0.878 \text{ Log } K_{ow} - 2.40$

  $n = 20$ $r^2 = 0.911$ $s = 0.335$

- $\text{Log LOEC (mol/L)} = -0.862 \text{ Log } K_{ow} - 2.16$

  $n = 20$ $r^2 = 0.913$ $s = 0.325$

- $\text{Log ChV (mol/L)} = -0.870 \text{ Log } K_{ow} - 2.28$

  $n = 20$ $r^2 = 0.914$ $s = 0.327$

- $\text{Log ChV (mg/L)} = \text{antilog ChV (mol/L)} * \text{mw}$

QSARS BETWEEN NEUTRAL ORGANIC CHEMICALS AND GREEN ALGAE TOXICITY (GROWTH) (Nabholz, in preparation)

- $\text{Log ChV (mmol/L)} = 0.036 - 0.634 \text{ Log } K_{ow}$

  $n = 6$ $r^2 = 0.99$

- $\text{Log 96-h } EC_{50} \text{ (mmol/L)} = 1.48 - 0.869 \text{ Log } K_{ow}$

  $n = 22$ $r^2 = 0.93$

Please note: The QSARs referenced here and elsewhere in the report are now available as a computer program called ECOSAR (EPA-748-F-93-002). Limited copies are available from the National Center for Environmental Publications and Information, U.S. EPA, 26 West Martin Luther King Drive, Cincinnati, OH 45268 (513-569-7985). In addition, copies may be obtained from:

- U.S. Government Printing Office, Superintendent of Documents, ATTN: Electronic Product Sales Coordinator, P.O. Box 37082, Washington, DC 20013-7082 (202-512-1530);
- Federal Bulletin Board, U.S. Government Printing Office of Electronic Information Dissemination Services (202-512-1524); or
- National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650) (order as computer program PB94-500485).

APPENDIX B

INPUT AND OUTPUT PARAMETERS FOR EXAMS II

INPUT PARAMETERS FOR EXAMS II

EXAMS II estimates exposure, fate, and persistence of an organic chemical after being released into an aquatic ecosystem (Burns, 1989). EXAMS II requires input of data into three files that describe the chemical, the environment, and the chemical loading to the environment.

Critical inputs to the chemistry file for this example were the water solubility, octanol-water partition coefficient (K_{ow}), soil/sediment organic carbon-water partition coefficient (K_{oc}), and biodegradation rate constant. These parameters are important in modeling the test chemical partitions between the water column and sediments. This particular analysis used a log K_{ow} of 6.56.

The environment file was culled from a set of predefined or canonical environments. Data including stream geometry and surface water flow rates are included here. Two important parameters that were user-defined in this example were the benthic and suspended sediment organic carbon content and the concentration of microorganisms in the sediments active in the biodegradation of the compound. The mass of test chemical released per unit time is entered into the loading file.

OUTPUT OF EXAMS II

The EXAMS II output includes tables summarizing test chemical properties; environmental characteristics; chemical loadings; steady-state mean, minimum, and maximum concentrations in various environmental compartments; and an exposure analysis summary (see example below).

EXAMPLE OUTPUT OF EXAMS (NOT CASE STUDY PMN)

Exposure (maximum steady-state concentrations):

Water column:	7.884E-03 mg/L dissolved; total = 8.255E-03 mg/L
Benthic sediments:	7.377E-03 mg/L dissolved in pore water; maximum total concentration = 33.1 mg/kg (dry weight)
Biota (µg/g dry weight):	Plankton: 7.68E+03 Benthos: 7.18E+03

Fate:

Total steady-state accumulation:	494 kg, with 0.29 percent in the water column and 99.71 percent in the benthic sediments.
Total chemical load:	27 kg/month. Disposition: 0.00 percent chemically transformed, 0.00 percent biotransformed, 0.00 percent volatilized, and 100.00 percent exported via other pathways.

Persistence:

After 16.0 months of recovery time, the water column had lost 65.82 percent of its initial chemical burden; the benthic zone had lost 60.08 percent; systemwide total loss of chemical = 60.1 percent. Five half-lives (>95 percent cleanup) thus require about 60 months.

SECTION TWO

ECOLOGICAL RISK ASSESSMENT CASE STUDY:

RISK ASSESSMENT FOR THE RELEASE OF RECOMBINANT RHIZOBIA AT A SMALL-SCALE AGRICULTURAL FIELD SITE

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DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
FA	fluorescent antibody
GEM	genetically engineered microorganism
MDL	minimum detection limit
MPN	most probable number
OPPT	Office of Pollution Prevention and Toxics
OTS	Office of Toxic Substances
PMN	premanufacture notice
RDM	rhizobia-defined medium
TSCA	Toxic Substances Control Act

ABSTRACT

This ecological risk assessment concerns a small-scale field test of genetically engineered *Rhizobium meliloti* strains. The strains were submitted in 1988 as part of a premanufacture notice (PMN) to the Office of Toxic Substances (OTS, currently the Office of Pollution Prevention and Toxics, OPPT) for tests to be conducted in 1989-1990. The rhizobia were genetically modified by the insertion of antibiotic resistance markers or by the addition of both antibiotic resistance and *nif* genes to enhance nitrogen fixation. *R. meliloti* form nodules and fix nitrogen in alfalfa (*Medicago sativa*), sweet clover (*Melilotus*), and fenugreek (*Trigonella*). The surrounding agroecosystem near Sun Prairie, Wisconsin, constituted the area of concern for ecological effects. Literature accounts of rhizobial movement, field test site characteristics, and field test design indicated that the microorganisms had only a minimal potential for migrating beyond the field test plot. The primary assessment endpoint examined during the small-scale field test was the potential for these recombinants to alter top growth of alfalfa. The ecological concerns for large-scale releases of recombinant rhizobia—such as increased growth of nontarget legumes, decreased growth of target legumes, spread of antibiotic resistance genes, nitrogen cycling disruption, and alteration of host range—were of low concern for the agroecosystem around the test site.

Actual data obtained from the small-scale field study confirmed the predictions in the OTS PMN risk assessment conducted in 1988 and those in this ecological risk assessment. Little horizontal, vertical, or aerial migration of *R. meliloti* occurred. The rhizobia primarily moved with the alfalfa root system. When compared with unmodified strains, recombinant rhizobia did not cause significant changes in nitrogen fixation, as measured indirectly by alfalfa yield. Recombinant strains did not out-compete parental strains, alleviating the concern for displacement of the indigenous rhizobia.

2.1. RISK ASSESSMENT APPROACH

This case study represents a typical risk assessment for a premanufacture notice (PMN) received by the U.S. Environmental Protection Agency's (EPA's) Office of Pollution Prevention and Toxics (OPPT). OPPT effectively evaluates the potential risk using the paradigm of "Risk = Hazard × Exposure" (Sayre, 1990). This paradigm is consistent with the *Framework for Ecological Risk Assessment* (U.S. EPA, 1992). Figure 2-1 demonstrates how the assessment was structured, using the framework report as guidance.

Since the framework report focuses on physical and chemical rather than biological stressors, the report does not adequately address certain aspects of this risk assessment. These include:

- the need for fate monitoring to build a data base of rhizobial behavior for larger-scale releases;
- the potential that in some cases the introduced deoxyribonucleic acid (DNA) might move from the genetically engineered microorganism (GEM) to other environmental recipients;
- consideration of field site design that limited microbial dissemination beyond the site, thereby alleviating the need for certain effects testing;
- the evaluation of exposure resulting from culturing and transporting GEMs to the field site; and
- construct considerations.

Reviewer Comments on Risk Assessment Approach

- *As currently formulated, EPA's Framework for Ecological Risk Assessment does not address fundamental differences between biological stressors and chemical and physical stressors. These differences include concerns unique to living entities, such as replication, colonization, and genetic evolution. This case study should provide a useful model for assessing risks of future limited releases of genetically engineered microorganisms in agroecosystems.*
- *This case study does not address more general ecological risks, nor does it consider risks of large-scale or commercial release of GEMs. However, the study does serve as an important ground-breaking document because risk assessments of GEMs released into agroecosystems will become more common in the near future.*

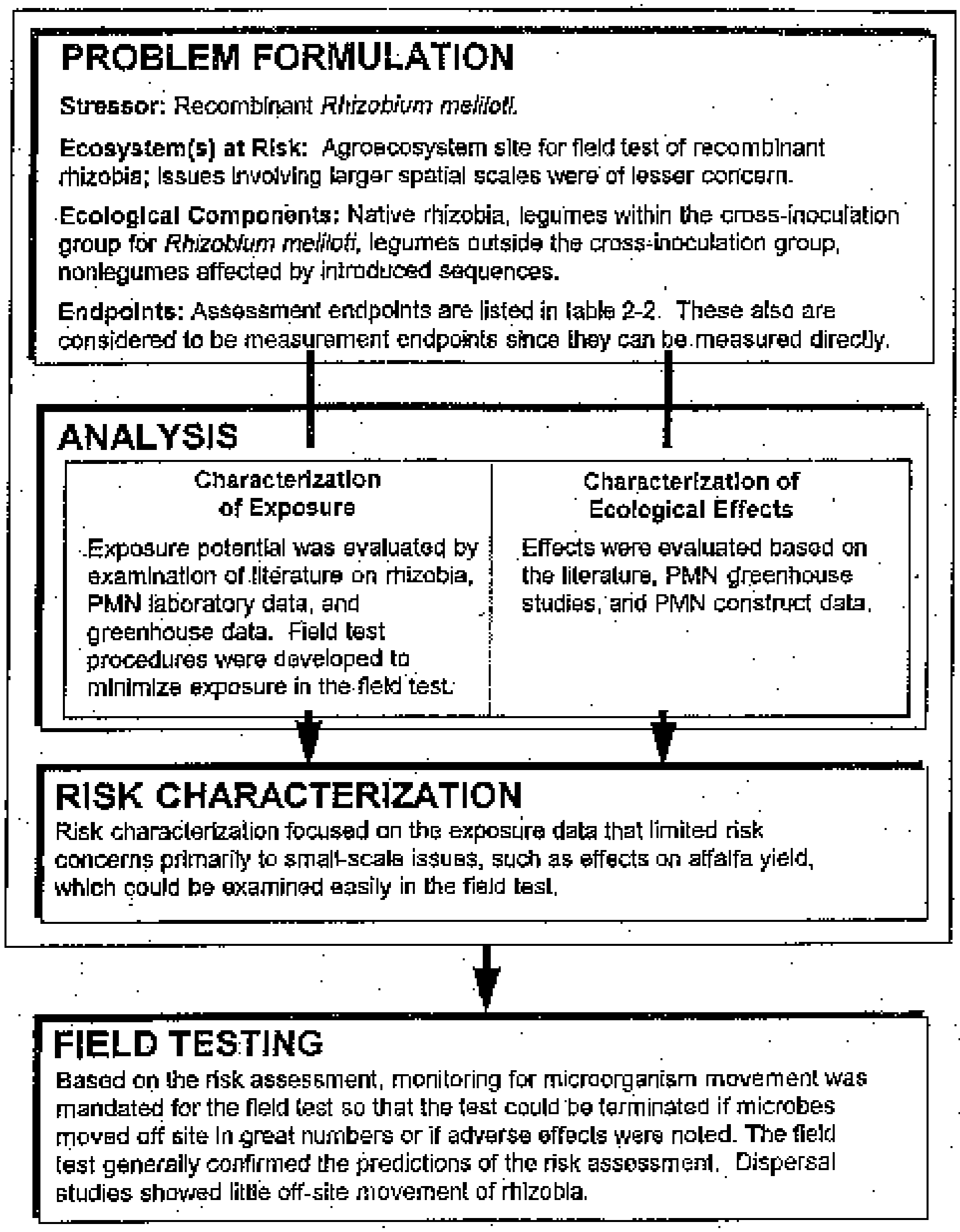

Figure 2-1. Structure of assessment for small-scale field tests with recombinant rhizobia

Reviewer Comments on Risk Assessment Approach (continued)

- *The reviewers did not consider the application of the framework to microbial stressors an insurmountable barrier. However, they agreed that both the framework report and similar future case studies should acknowledge the unique properties and complexities of a living, changing stressor. The reviewers suggested that applying the framework to biotechnological risks—such as the release of GEMs—might require providing the framework audience with the biotechnological details needed for appraising the relative importance of the risk factors.*

2.2. STATUTORY AND REGULATORY BACKGROUND

The "Coordinated Framework for Regulation of Biotechnology" (Office of Science and Technology Policy, 1986) explains that the Toxic Substances Control Act (TSCA) gives EPA the authority to review certain classes of biotechnology products. Under the coordinated framework, biotechnology products are regulated in accordance with the use of each product (Milewski, 1990). Uses of microorganisms not covered by other existing authorities (U.S. Department of Agriculture, Food and Drug Administration, EPA's Office of Pesticide Programs) are reviewed by EPA's OPPT under TSCA; thus, TSCA serves as a "gap-filling" statute. TSCA's applicability follows from the interpretation that microbes are chemical substances under TSCA. Candidates for review are limited to those commercial microorganisms that have been altered to contain genetic information from dissimilar source organisms. EPA describes as dissimilar those organisms produced using DNA from different taxonomic genera. Such microorganisms are considered "intergeneric." EPA does not regulate the use of naturally occurring rhizobial inoculants.

TSCA applies only to products developed for commercial purposes, whether for contained systems or environmental releases. Under Section 5 of TSCA, manufacturers and importers of intergeneric microorganisms must submit a PMN at least 90 days prior to beginning manufacture or import. Under TSCA authority, OPPT can require information on microbial biotechnology products in order to identify potential hazards and exposures. OPPT also can require testing a microbial biotechnology product that may present an unreasonable risk of injury to human health or the environment or that is produced in substantial quantities and may result in substantial environmental release or substantial human exposure. Finally, OPPT can restrict the production, processing, distribution, use, and disposal of a microbial biotechnology product if it presents an unreasonable risk of injury to human health or the environment.

Because TSCA applies only to microorganisms developed for commercial purposes, EPA currently requests that industry voluntarily comply with the PMN reporting requirements for any commercial research and development field test that involves the release of intergeneric microorganisms involving a TSCA use. As a result, the PMN submission for the small-scale field test of genetically engineered strains of *Rhizobium meliloti*, the subject of this ecological risk assessment, was submitted on a voluntary basis by Biotechnica Agriculture, Inc., in 1988. Approval of the field test resulted in the issuance of a 5(e) Consent Order, which bound the company to the protocols, monitoring procedures, and data collection approved by EPA.

2.3. CASE STUDY DESCRIPTION

2.3.1. Background Information and Objective

This case study focuses primarily on the small-scale field testing of four recombinant strains of *R. meliloti*. Rhizobia, a general term for various species of the genus *Rhizobium*, are Gram-negative, motile, rod-shaped, aerobic bacteria that infect legume roots. A symbiotic relationship forms in which the bacteria fix atmospheric nitrogen, providing ammonium for protein production in the plant. In exchange, the bacteria obtain energy from the plant in the form of photosynthate, specifically dicarboxylates.

The various species and biovars of *Rhizobium* have been designated according to the types of legume plants they infect, such as alfalfa, clovers, beans, vetch, or lotus. The specificity of infection by certain species or biovars of *Rhizobium* has led to the loose designation of "cross-inoculation" groups (Alexander, 1977). For example, the alfalfa group consists of *R. meliloti*, which is capable of infecting not only alfalfa (*Medicago*), but sweet clover (*Melilotus*) and fenugreek (*Trigonella*).

The symbiotic relationship between rhizobia and legumes is of great importance in agriculture, as legumes typically are not fertilized with nitrogen if rhizobia are present. In fact, high nitrogen contents in soils actually suppress nitrogen fixation by the nodules. More important, symbiotic nitrogen fixation contributes greatly to the nitrogen cycle. In association with alfalfa, rhizobia fix nitrogen vigorously, perhaps fixing between 125 and 335 kg of nitrogen per hectare each year (Alexander, 1977).

This case study has two purposes. First, the case study will examine the information submitted and used during the OPPT risk assessment to determine whether the framework assessment process can use the information as efficiently. Second, the case study will examine the data generated from the field to determine how accurately the risk assessment process predicted risks associated with the field test.

The area of concern for possible adverse ecological impacts is the surrounding agroecosystem. Ecological concerns are contingent on the ability of the rhizobia to survive and spread beyond the immediate area of the field site.

2.3.2. Problem Formulation

2.3.2.1. Planning

This risk assessment focused on determining the potential adverse effects of conducting a small-scale field test with recombinant rhizobia. However, the data gathered from the field site also may prove useful in projecting adverse effects that could result from a large- or commercial-scale release. Before such a release occurs, the potential ecological effects for a large-scale release need to be addressed.

The PMN submission supplied laboratory data on the microorganism identity, construct information, and microorganism characteristics and behavior. Greenhouse data addressed the effects on alfalfa (yield data), survival, and competitiveness of the recombinant strains (nodule occupancy). These data contributed to the decision-making process for approval of the small-scale field test. Field test protocols and a site evaluation conducted prior to the field tests also helped reach the decision to approve the small-scale field test of these recombinant rhizobia.

Figure 2-2 illustrates the various components of the risk assessment process for PMNs, from the data submissions through the decision-making processes prior to approval for commercial release. The field test design included several approaches to evaluating adverse effects from small-scale releases of recombinant rhizobia. Yields of alfalfa were an indirect measure of changes in the nitrogen-fixing ability of rhizobial strains. Nodule occupancy indicated competitiveness of the rhizobial strains with the indigenous rhizobial populations. The test design also included plans to measure the persistence of the microorganism in the rhizosphere. Finally, to test the prediction that only limited dissemination of the recombinant microorganisms beyond the site would occur, the test design included monitoring both soil and air for the presence of these microorganisms.

2.3.2.2. Stressor Characteristics

The primary stressors in this case study are the recombinant rhizobia, as opposed to chemical or physical stressors. In this study, the stressor has the potential to split into subcomponents of a biological nature (pathogenicity, altered legume growth resulting from the microbe) and subcomponents of a chemical nature (production of toxins, detrimental metabolites, and overproduction of nitrate).

As with chemical stressors, characterizing the recombinant microbes to predict their potential adverse effects constitutes a critical component for the risk assessment. For the recombinant microorganism, characterization includes a description of the donor and recipient microorganisms, including their taxonomic derivation. The phenotypic traits of most GEMs reviewed in OPPT are encoded and analyzed with a PC-microcomputer version of the "Micro-IS" software package; this data system was originally developed by the National Institutes of Health (Segal, 1988). A description of the techniques used to construct the PMN microorganism also contributes to characterization of the GEM.

The final step for GEM identification involves verifying that GEM DNA contains the DNA of interest, along with additional vector DNA. This analysis is based on PMN submission information that usually includes the following:

- construction of the DNA cassette that codes for traits such as enhanced nitrogen fixation;
- a complete description of the integration site in the *R. meliloti* genome;
- construction of the vector containing the cassette;
- introduction of the vector carrying the cassette into the recipient microorganism; and
- final construct and genetic stability of the PMN microorganism.

OPPT reviewed data from restriction digests, DNA probe verification, and phenotypic analysis of recombinants for this step of GEM characterization. OPPT also used DNA sequence data bases and software such as GENEMBL and DNA Star to examine introduced sequences. These sequences are examined to determine functions of identified DNA and the potential for unidentified DNA sequences (such as open reading frames) to encode known protein products.

Table 2-1 summarizes the recombinant rhizobia tested in the 1989-1990 field tests. The genes inserted in the four recombinant strains were added to the same insertion site, the *ino* site. Note that each wild-type *R. meliloti* recipient contains the usual complement of *nif* genes necessary for nitrogen fixation.

Table 2-1. Table of Recombinant Rhizobia for 1989-1990 Field Tests

PMN No.	Biotechnica Agriculture, Inc., Strain	Recipient	Modification	Insertion Site
P88-1116	RMB7101	RCR2011[a]	*omega*/strep/spec	*ino*
P88-1118	RMB7201	PC[b]	*omega*/strep/spec	*ino*
P88-1120	RMB7401	UC445[c]	*omega*/strep/spec	*ino*
P89-280	RMB7103	RCR2011	*omega*/strep/spec/*nif*	*ino*

[a] Streptomycin-sensitive parent of *R. meliloti* strain Rm1021. Strain Rm1021 is a spontaneous streptomycin-resistant mutant arising from strain RCR2011. Strain RCR2011 is derived from a natural isolate, strain SU47 (Rothamsted Experimental Station collection).

[b] Natural isolate obtained in 1986 from a root nodule of inoculated alfalfa plant grown in soil from the Chippewa Agricultural Station, Pepin County, Wisconsin.

[c] California soil isolate (UC445 or CA445) effective in the alfalfa cultivar Hairy Peruvian.

Each introduced sequence of the constructs was examined for the potential to cause adverse impact. The insertion site, too, may disrupt recipient DNA. The altered or added DNA sequences are noted below, along with their potential ecological impact (see figure 2-3 for additional information about constructs):

***ino* insertion site.** The *ino* sequence encodes genes responsible for the metabolism of myoinositol, a substrate usable as a carbon source during saprophytic growth. If an introduced DNA sequence inactivates genes at this insertion site, rhizobia would have decreased survival in soil and senescing plant roots. No other adverse effect would be expected.

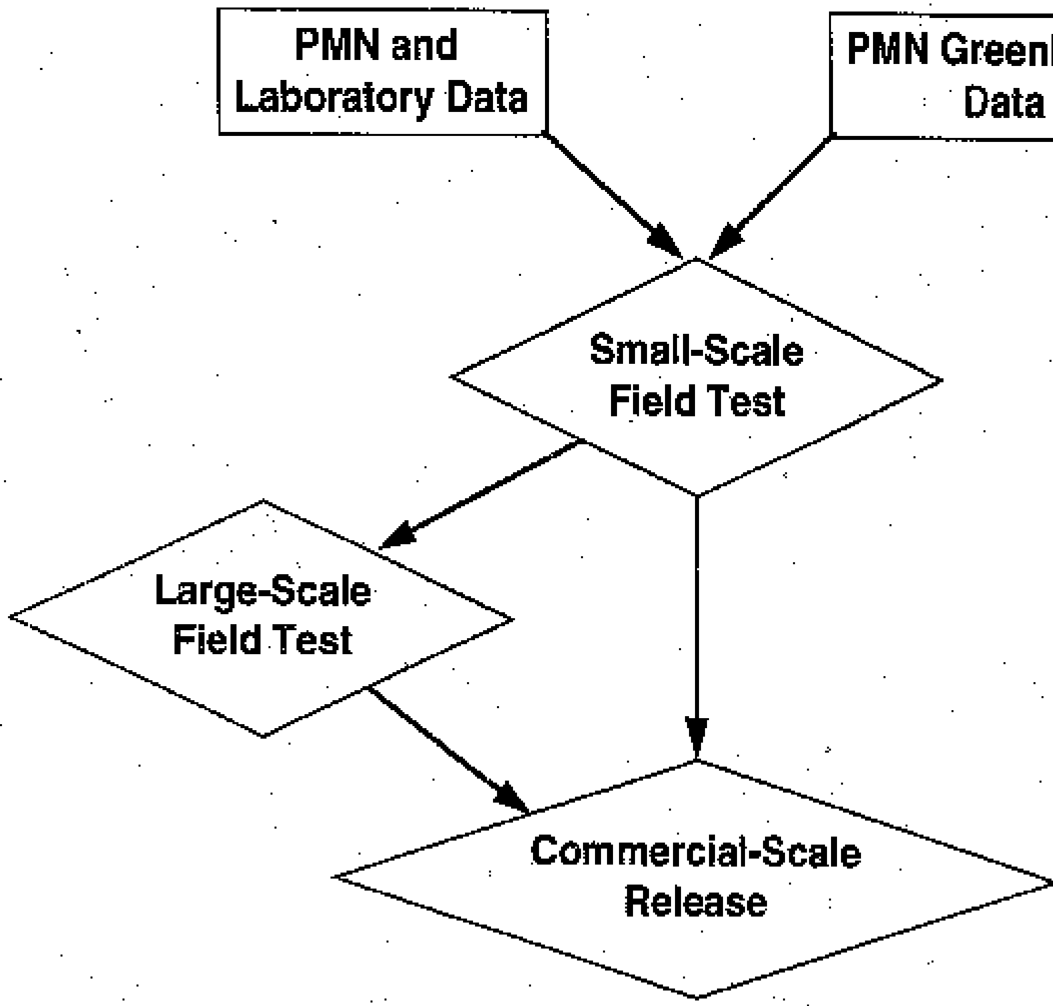

Figure 2-2. PMN data/information, components, and decisions made

ino*/T_1T_2/*nifD* promoter/tet^R/*nifH* leader/*nifA*/*omega*/*ino

ino = inositol region of *R. meliloti* located on megaplasmid pRmeSU47b

T_1T_2 = transcriptional terminator sequences from the *rrnB* gene that encodes the 5S rRNA of *Escherichia coli*

nifD promoter = promoter derived from *Bradyrhizobium japonicum*

tet fragment = 200 bp of the *tet*^{R} gene derived from pBR322

nifH = synthetic 21 bp oligomer linker with two added restriction sites plus the *R. meliloti nifH* DNA that encodes untranslated leader RNA of *nifH* gene

nifA = *R. meliloti nifA* gene

omega = gene constructed by Prentki and Krisch (1984) derived from plasmid R100 originally isolated from *Shigella flexineri*; encodes a transcriptional terminator and resistance to streptomycin and spectinomycin

Figure 2-3. Cassette diagram for RMB7103 with only primary sequences added (serves as illustration of a gene cassette introduced into the *ino* site)

- **T_1T_2, *nifH*.** Because the T_1T_2 termination sequence halts the transcription of the introduced DNA into messenger RNA, this sequence limits the effects of the cassette on surrounding DNA. Consisting of only a leader sequence for the *nifA* and other genes, *nifH* has little likelihood of causing adverse ecological effects.

- ***nifA nifD.*** A regulatory gene, the *nifA* sequence controls the production of the nitrogenase enzyme, which brings about nitrogen fixation in alfalfa. Altered nitrogenase production could lead to decreased growth of alfalfa or increased growth of weedy relatives. The same concerns apply to the cassette's promoter sequence, *nifD.*

- ***tet, omega.*** The *omeg*a fragment encodes resistance to streptomycin and spectinomycin. Transfer of these genes from GEMs to human or animal pathogens would render them resistant to streptomycin and spectinomycin, but such resistance transfer is not of concern in small-scale field trials. The *tet* gene does not encode resistance to tetracycline because it is only a gene fragment.

Essentially, the construct analysis narrowed the concerns about effects to the potential for decreased yield in the target legume, alfalfa. The construct analysis did not eliminate concerns for increased competitiveness or survival of the recombinant rhizobia relative to the wild-type strains.

The fate of the introduced DNA in the GEM is an ecological concern. Natural gene transfer of this DNA from recombinant strains to environmental receptors could produce secondary stressors. However, in the case of these GEMs, careful analysis of the constructs, available literature, and laboratory data indicated little need to monitor for the existence of secondary stressors. For example, under optimal laboratory conditions, genetic transfer of the megaplasmid containing the insertion point was not detected at a detection limit of 10^{-8} (Finan et al., 1986). These constructs cannot transfer by means of transposition, because the *omega* fragment lacks transposition functions. Finally, data on RMB7101 indicated a reversion frequency to a streptomycin-sensitive phenotype of less than 6.3×10^{-8} (Sayre, 1988).

2.3.2.3. Ecosystem Potentially at Risk

The field test plots consisted of less than 1 acre in the northwest corner of a 14-acre parcel leased from a 39-acre farm. This farm is located in Dane County, Wisconsin, directly north of the city of Sun Prairie and 12 miles east of Madison. Sparsely populated agricultural land lies to the north and east of the site. Residential areas lie within a mile to the south and 1.5 miles to the west. Dane County is approximately 80 percent farmland, with approximately 80 percent of this land in crops: corn for grain and silage, alfalfa, other hay, oats, sweet corn, and soybeans.

Potential biotic components of the agroecosystem include target and nontarget legumes (including weedy legumes), rotational nonlegume crops, native rhizobia, and bacterial pathogens that can acquire antibiotic resistance genes from the recombinant rhizobia (table 2-2).

Table 2-2. Linkages Among Assessment Endpoints and Data Needs Relevant to Endpoint Evaluation

Assessment Endpoint		Predictive Risk Assessment (information used)[a]	Endpoints Monitored in Small-Scale Test	Future Large-Scale Issues
1.	Decreased alfalfa growth	GH	X	X
2.	Decreased growth of legumes outside cross-inoculation group	TX, CA		X
3.	Decreased growth of nonlegume crop plants	GH, CA, TX		X
4.	Unanticipated effects of introduced DNA sequences	CA		
5.	Effects of introduced DNA on recipient DNA at insertion site	CA		
6.	Unanticipated effects of recipient microbe	TX		
7.	Effects of antibiotic resistance genes	BSAC		
8.	Competitive displacement of native rhizobia if coupled with any hazards listed in 1-3 or 9-10		X	X
9.	Increased/decreased growth of sweet clover			X
10.	Increased/decreased growth of fenugreek			X
11.	Effects of coumarin on cattle			X
12.	Effects on nitrogen cycle			X

[a] Legend:
BSAC =addressed by the EPA Subcommittee of the Biotechnology Science Advisory Board
CA = addressed by construct analysis
GH = addressed by PMN greenhouse data
TX = addressed by taxonomic analysis of recipient rhizobia

Displacement of the indigenous rhizobia by recombinants also may alter ecosystem structure. Such a change would adversely affect the ecosystem if the constructs have a lower nitrogen-fixing capacity than native rhizobia. For example, *Bradyrhizobium japonicum* strain 123, which fixes nitrogen poorly in the field, has out-competed and displaced native strains in the Midwest, resulting in decreased nitrogen fixation in soybean plants (Tiedje et al., 1989). Displacement of native rhizobia by recombinants having a greater nitrogen-fixing capacity also has the potential to affect ecosystem function adversely. Increased soil nitrogen might disrupt the nitrogen cycle balance or lead to localized pollution of ground water by nitrates.

2.3.2.4. Endpoint Selection

The assessment endpoints reflect the delineation of the ecosystem at risk: primary concern focused on the area immediately surrounding the field plot, with some lessening concern for areas farther removed from the field site. If the monitoring of the microorganisms during the field test had shown significant off-site movement and spread (particularly if linked with decreased alfalfa growth in the field or other adverse effects), then the field test would have been terminated and the risk assessment expanded to include larger-scale issues.

In this ecological risk assessment, many of the 12 assessment endpoints listed in table 2-2 can be measured directly, eliminating the need to identify measurement endpoints for these assessment endpoints. Table 2-2 links the assessment endpoints to the data needed to evaluate them. Data relating to assessment endpoints originate from four sources: the literature, laboratory studies, greenhouse studies, and the field test. Literature information and laboratory studies conducted for the PMN can at least partially address assessment endpoints 4 through 7 of table 2-2. Greenhouse studies for the PMN provide information on assessment endpoints 1 and 3, while the small-scale field test concerns assessment endpoints 1 and 8. Assessment endpoints 1 through 3 and 8 through 12 concern information needed prior to large-scale release. Table 2-3 links the GEMs to monitoring and data needs. field test

Table 2-3. Linkages Among Stressor, Monitoring, and Data Needs Relevant to Endpoint Evaluation

Exposure Element[a]	Risk Assessment (information used)[b]	Measurement Endpoints for Small-Scale Test	Future Large-Scale Issues
Detection of GEM in nodule	GH	X	
Survival of GEM in soil, rhizosphere	GH,L	X	
Monitoring of GEM in soil, air, water	F	X	
Monitoring of gene transfer	L, CA		X

[a] Presence of the GEM or the introduced DNA in various media is necessary for linking the GEM with the assessment endpoints in table 2-2.

[b] Legend: CA = addressed by construct analysis
F = addressed by examination of field prior to GEM release
GH = addressed by PMN greenhouse data
L = addressed by PMN laboratory data

concerns assessment endpoints 1 and 8. Assessment endpoints 1 through 3 and 8 through 12 concern information needed prior to large-scale release. Table 2-3 links the GEMs to monitoring and data needs.

Comments on Problem Formulation

General reviewer comments:

- ! *The following factors in the current case study set it aside from risk assessments of chemical and physical stressors:*

  - -- *the unique complexities of a microbial stressor;*
  - -- *the real and imagined risks of genetically engineered microorganisms; and*
  - -- *detecting off-site migration.*

- ! *As risk assessment experience for microbial stressors accumulates, risk assessors will gain facility in addressing these factors. The process should result in an enhanced knowledge base that can feed back into the risk assessment process itself and can be implemented in the education of scientific and regulatory communities as well as the general public.*

Comments on Problem Formulation (continued)

- ! *Releases of genetically engineered rhizobia are probably the best available model for initial release of GEMs. Thus, although supporting data for low risk of small-scale field tests were weak (compromised or poorly designed greenhouse studies), other factors contributed to the decision to issue consent for the study, including:*

  - -- *the knowledge base on the generally innocuous nature of the rhizobia, e.g., the history of their application worldwide in the enhancement of symbiotic nitrogen fixation;*
  - -- *site characteristics that would tend to inhibit spread of the introduced strains; and*
  - -- *the nature of the genetic construct in the GEMs.*

Comments on Problem Formulation (continued)

- *The case study set criteria for termination of the test and described monitoring procedures. The authors may wish to point out the extent to which exotic rhizobia have already been introduced in the United States and the consequences. The authors do describe how the introduction of a highly effective and competitive* Bradyrhizobium japonicum *(strain 123) became problematic, out-competing indigenous rhizobia with a greater capacity for nitrogen fixation. The effectiveness data, which addressed this particular kind of risk, proved inconclusive. Although most introduced rhizobia have been harmless, the legume kudzu (and its symbiotic rhizobia) has become an infamous pest in the southern United States.*

- *In the characterization of the stressors, the authors split risks of the GEMs into* ***biological*** *(i.e., pathogenicity, altered legume growth, microbial competition, gene release) and* ***chemical*** *(i.e., toxins, detrimental metabolites such as nitrate). This approach appears useful in addressing risks of plant-associated microbes.*

- *One category of secondary stressors consists of microbial recipients that could acquire introduced DNA by natural gene transfer, such as through bacteriophages or conjugation. Several reviewers pointed out that the antibiotic marker genes were likely to be of greater concern than the* nif *genes themselves.*

- *The review panel expressed interest in whether risk assessment for microbial releases into an agroecosystem also should consider risk in the broader context of general ecological effects; that is, should a more general range of nontarget animals, plants, microbes, or ecosystem function be incorporated as measurement endpoints for the general health of the ecosystem? The case study focuses on decreased production in a commercially important crop as opposed to effects on surrounding ecosystems. However, a risk to surrounding ecosystems may not be a risk to the agroecosystem. Although the study discussed broader ecological risks outside the field site, the study considered the risk of exposure beyond the site as minimal.*

Comments on Problem Formulation (continued)

Authors' comments:

- *At the time the Agency reviewed this proposed field test (1988), it was not deemed necessary to assess risk in the broader context of general ecological effects such as the general range of nontarget animals, plants, microbes, or ecosystem function for several reasons. First, the field test was a small-scale test that was expected to remain small scale given the data available in the literature on rhizobial movement and specific site characteristics for this test. Second, there was no reason to expect that the genetic modifications made to the recipient rhizobial strains would result in any broad ecological consequences. The genetic alterations of (1) enhancing the existing trait of nitrogen fixation and (2) insertion of antibiotic resistance genes to serve as markers for detection were not expected to confer on these microorganisms the trait of pathogenicity to plants or animals, nor to alter the host range of plants these rhizobia can infect (nodulate). Competitiveness of the recombinant rhizobia relative to the parental strains and indigenous rhizobia was addressed in the field studies. Similarly, in 1988, standardized validated protocols for assessing disturbances in ecosystem function were not available. Currently, the processes of (1) identification of ecologically significant endpoints for assessing ecosystem function, (2) the development of protocols/methodology for assessing those endpoints, and (3) the interpretation of results from such tests are all still in their infancy.*

- *Because EPA's framework report did not address several aspects of an ecological risk assessment relevant to biological stressors, addressing these aspects proves difficult. In this ecological risk assessment, two key facets in particular were difficult to address: (1) the need to identify construct issues in general and to use the construct information to lessen the concerns for fate and effects and (2) the need to monitor the movement and survival of the GEMs in different media. The exposure elements in table 2-3 were critical to identifying the ecosystem at risk, but including the table proved problematic within the context of the framework guidance.*

- *In addition, it was difficult to decide which table 2-2 endpoints to list and whether these endpoints were assessment or measurement endpoints in this particular case study.*

2.3.3. Analysis: Characterization of Exposure

The exposure profile in this case study can contain specific information because both the intended number of microorganisms to be applied and the area of application are known. The test specified applying microorganisms by means of in-furrow spraying at the time of planting. The test applied a total of approximately 6×10^{12} microorganisms to approximately 0.8 acre. The first release, which occurred on May 24, 1989, consisted of 5.52×10^{12} cells for a strain comparison test. The second release, which took place on May 25, 1989, contained 5.52×10^{11} cells for a strain competition test. Monitoring these microorganisms continued for 2 years, collecting yield data for alfalfa over two growing seasons. Post-termination monitoring of recombinant rhizobia in soil extended months beyond the last alfalfa harvest.

Although the initial exposure is well characterized, uncertainty regarding exposure over time arises as a result of microbial death, reproduction, and transport. Considerations include survival in soil and root nodules and dissemination away from the planted rows within and beyond the field plot as a result of vertical and horizontal movement through the soil or through wind-vectoring of aerosolized microbes.

2.3.3.1. Stressor Characterization

Laboratory studies showed that recombinants in bulk field soil underwent a 1-log reduction in survival over a 4-week period. Literature on rhizobial survival in soil available at the time of the PMN review indicated that only limited horizontal and lateral movement of rhizobia in soil would occur. Three studies indicated that lateral movement by wind, water, and bacterial motility was on the order of only 2.5 to 5 cm (Kellerman and Fawcett, 1907; Robson and Loneragan, 1970; and Brockwell et al., 1972, as cited in Madsen and Alexander, 1982). However, some of these studies have limited utility as a result of their qualitative nature, use of autoclaved soil, or lack of proper controls.

Aside from survival as intact cells in soil, rhizobia exist in a morphologically altered form (bacteroids) in root nodules. These intranodal rhizobia can survive saprophytically at the end of the growing season, when the alfalfa senesces. These populations can then reinfect alfalfa in the field the following year. Consequently, the rhizobial population in the soil shows seasonal variations.

The present case study needed to determine how well the recombinants could be monitored in the field. The 1988 PMN presented minimum detection limits and recovery efficiencies for the rhizobial strains, based on the technologies available at that time. At the time of this review, EPA considered the use of selective antibiotic media as the appropriate method for monitoring rhizobial numbers in this small-scale field test.

In the data submitted with the PMN, the company reported that use of selective antibiotic media gave an actual minimum detection limit (MDL) between 2×10^4 and 2×10^5 cells/g soil. The use of fluorescent antibody (FA) technique lowered the MDL to 10^3 to 10^4 cells/g soil. Appendix A provides additional details of the monitoring and enumeration techniques.

In circumstances with low rhizobial counts, such as horizontal dispersal studies, field tests may require a more sensitive detection limit. To meet this need, a most probable number (MPN) enumeration procedure was developed. This MPN technique involved placing alfalfa plants in growth pouches and infecting their roots with dilutions of soil suspensions. Any plants in which at least a single nodule formed was scored as a positive. In some cases, laboratory personnel identified the rhizobial strain present in nodules on the plants exposed to the highest dilution that resulted in nodule formation. Although the MPN technique inherently has a high statistical error, it gives a minimum detection limit of approximately 10 cells/g soil using soil from the field test site. The MPN technique was more sensitive but less quantitative than the other enumeration methods.

Prior to the field tests, the MPN technique was used to determine the number of indigenous rhizobia in the field site soil. At the Sun Prairie site, the number of indigenous rhizobia was <10 rhizobia/g soil.

The PMN submission included both routine and emergency termination procedures, which received EPA approval prior to the field studies. Routine termination procedures after completion of the field tests included plowing under the test plots and, if necessary, applying glyphosphate herbicide to kill any remaining alfalfa or weeds. Severe adverse effects such as die-off of the alfalfa, tremendous increases in population density, or movement off-site would indicate a need for emergency termination. Emergency procedures included treatment of the test area with methyl bromide to minimize the microbial populations. EPA did not specify the exact criteria that should have triggered emergency termination procedures. Instead, EPA advised the company to report any "irregularities."

2.3.3.2. Ecosystem Characterization

The ecosystem under consideration was a 0.8-acre (275 ft. × 300 ft.) field site plus the immediate surrounding agroecosystem in Dane County, Wisconsin (with lesser concern for areas farther removed from the site itself). The test site lay 500 feet from a road and was separated from it by a fence. The majority of the field was Plano silt loam that consisted of deep, well- to moderately drained soil on glaciated uplands. The soil contained high levels of phosphorus, potassium, magnesium, manganese, iron, zinc, and copper. Organic matter was 3.3 percent, and pH was 6.8. Organic nitrogen content was not supplied, but was roughly estimated at 0.19 percent.[2] In a later PMN submission, the company stated that a nitrogen content of 0.20 percent was limiting for alfalfa growth.

The slope of the field was approximately 2 percent from east to west. Dane County receives approximately 31 inches of rain each year. Although infrequent, some runoff from the test plot was expected. The runoff would drain into a ditch south of the site and then enter a culvert that empties into Koshkonong Creek and eventually into Koshkonong Lake. The study did not monitor microorganisms in runoff water because their level was expected to be below detection limits. The site area had no wells that could become contaminated by dispersing microorganisms.

To address concerns that *R. meliloti* might infect nontarget legumes, the 14-acre test area and the ditch separating the test area from the road were scouted before and during the field trials for the presence of *Melilotus* (sweet clover) and weedy *Medicago* species.

2.3.3.3. Temporal Analysis

The field trials ran for a maximum of 2 years, but the company reserved the option to terminate the trials earlier if it so desired.

2.3.3.4. Exposure Analyses

To determine the spatial and temporal distributions of the GEMs at the field site, several studies were performed before the field test. These included laboratory studies on survival of the GEMs in pure culture, survival in soil, survival in rhizosphere soil in the greenhouse, and the ability of the GEMs to infect alfalfa in greenhouse studies.

The PMN included laboratory survival data of several recombinant rhizobial strains in soils. Unfortunately, the studies employed soils obtained from areas other than the test site. In addition, some of the studies failed to include parental strains as controls. *R. meliloti* strains RMB7101 and RMB7201 showed a 1- to 2-log reduction in numbers over a period of 6 weeks in both Chippewa soil and in soil obtained from another field. Later studies tested a streptomycin-resistant spontaneous mutant of RCR2011 against the four recombinant strains used in the field tests. Approximately a 1-log reduction in numbers for all the strains occurred over 4 weeks, with no significant difference between strains.

The PMN submission contained some data concerning the persistence of the recombinant *R. meliloti* in the rhizosphere. The rhizosphere samples were separated into two fractions, the inner and outer rhizospheres. Soil aggregates that fell off the roots with vigorous shaking represented the outer rhizosphere, while the inner rhizosphere consisted of the remaining root system and associated soil. Recombinant rhizobia persisted in both soil fractions and in nonrhizosphere soil, with only slight declines in numbers over the 3-week study.

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[2]As a very rough estimate of organic nitrogen levels, one may assume a conversion factor of 1.724 between organic matter and organic carbon (Broadbent, 1965). Therefore, the organic carbon content should be approximately 3.3 percent/1.724 = 1.9 percent organic carbon. Most agricultural surface soils have C:N ratios of approximately 10:1 (Bremner, 1965), suggesting that the soil had a nitrogen content of approximately 0.19 percent.

The PMN included two pilot tests of nodule occupancy to study competitiveness of the rhizobial strains. Competitiveness, in this context, means the ability to form nodules in alfalfa roots when competing with another *Rhizobium* strain. The presence of a strain in a nodule suggests that it is the strain that caused the nodule to form. In one study, the two parental strains, RCR2011 and PC, showed no significant differences in competitiveness when inoculated into alfalfa in a 1:1 ratio. The second test indicated no significant difference in competitiveness between a naturally occurring and a recombinant strain that was not one of the stressors in the test study.

EPA recommended including nodule occupancy as part of the field trials because of the absence of greenhouse nodule occupancy data for the GEMs in the field test. Also, nodule occupancy data can link altered alfalfa yield with the recombinant rhizobia. The field data on nodule occupancy showed no significant differences in nodule occupancy between the recombinant and the wild-type rhizobia (appendix D).

Monitoring the microbe at the field site can indicate whether the GEM is associated with changes in alfalfa yield and can track GEMs beyond the field site. The study monitored vertical, horizontal, and aerial dispersal of the recombinant rhizobia by means of the strain comparison test, described in appendix C.

Analysis of exposure also included a strain comparison test to determine the efficacy of the inoculants.

Comments on Characterization of Exposure

General reviewer comments:

- ! *The technology for monitoring the spread of introduced strains from the inoculation site suffered from potential limitations of sensitivity and specificity. Newer, more sensitive and highly specific technologies (e.g., polymerase chain reaction [PCR] amplification of strain-specific sequences, strain-specific probes, marker cassettes) could be brought to bear on these problems. The manufacturer also could provide quality assurance/quality control of the methods used to monitor these important endpoints (e.g., proper controls, background levels of native rhizobia).*

- ! *Further work on developing the idea of meaningful estimates of exposure to a microbiological stressor is needed. An examination of the uninoculated alfalfa border plants for nodule occupancy by strains introduced within the field plots might give another indication of their spread.*

Authors' comments:

- ! *Newer, more sensitive methods such as gene probes, PCR, or marker cassettes for detection of microorganisms in environmental samples have been developed in recent years. However, at the time of this submission, in 1988, those techniques were not routine laboratory analyses, and these laboratory research techniques have just recently been refined for use in environmental matrices. The use of antibiotic-selective media, supplemented with the fluorescent antibody technique, and the use of the MPN growth pouch technique were deemed appropriate by the Agency at the time of the review. The company was not required to submit actual QA/QC documents, but its use of appropriate methods and protocols, the use of proper controls as well as other aspects of its field experimental designs, and determination of background levels of rhizobia was reviewed by the Agency before the field test.*

2.3.4. Analysis: Characterization of Ecological Effects

2.3.4.1. Evaluation of Effects Data

The primary effects data reviewed prior to the field test consisted of greenhouse studies that examined alfalfa yields resulting from infection with recombinant rhizobia. For these studies, plants were grown in sterile vermiculite inoculated with RMB7103. Because vermiculite is nitrogen limiting, nodule occupancy data were probably not needed to show a causal link between rhizobia in the nodules and top growth of plants. One study demonstrated that no significant difference occurred in the growth of alfalfa plants inoculated with the parental strain, RCR2011, and a recombinant strain, RMB7101. Similarly, no significant difference in alfalfa yield occurred for plants inoculated with parental strain RCR2011 or recombinant strain RMB7103. In one study, recombinant strain RMB7103 gave a yield increase of 7.0 percent compared with RMB7101. Another study using these same recombinant strains showed no significant difference in their effect on alfalfa yield. Field yield studies also showed a lack of significant yield effects (appendix F).

However, the results of the greenhouse yield data were questionable for two reasons. First, the studies reported data as fresh weight of alfalfa top growth rather than as dry weight. Secondly, the studies were of short duration. Harvest of the alfalfa plants occurred 3 weeks after planting, but it usually takes 11 days for nitrogen fixation to begin. Consequently, these data demonstrated growth for only 10 days after the onset of nitrogen fixation in the nodules, making it difficult to interpret the effects of the inserted genes.

The study did not collect data on the growth of sweet clover or fenugreek, nor did the 1989-1990 field test generate data on effects on nonlegumes or on legumes outside the cross-inoculation group. A 1987 PMN offered limited qualitative information that indicated a lack of effects on such plants. The earlier PMN greenhouse studies exposed soybeans, peas, tender green beans, and clover to inoculation levels of 10^9 rhizobial cells/g of soil. Results indicated no adverse effects. Similarly, corn and ryegrass, crop plants commonly grown in rotation with alfalfa, showed no adverse effects from such exposures.

2.3.4.2. Evaluation of Causal Evidence

This section evaluates the strength of the relationship between the stressor and the measurement endpoint, yield of alfalfa. Problems associated with the greenhouse tests are noted in this section. In addition to the problems already noted for the greenhouse studies, extrapolating from the greenhouse to the field also presents difficulties. For example, such an extrapolation must take into account that the greenhouse and the field differ in climate, soil, and pest species.

2.3.4.3. Effects Needing Study in the Event of Significant Off-Site Migration or Large-Scale Release

This risk assessment assumed that only limited off-site migration of the rhizobia would occur. If, however, this risk assessment had been conducted for large-scale releases or if large numbers of rhizobia moved off-site, the risk assessment would need to address at least six main ecological concerns.

- *Increased competitiveness.* If large numbers of rhizobia moved off-site, then the risk assessment would need to examine whether the increased population resulted from enhanced competitiveness relative to native rhizobia. Displacement of native rhizobia by increased competitiveness would be a concern if the GEM decreased the growth of alfalfa or increased the growth of weeds.

- # *Increased nitrogen production.* Increased nitrogen production by alfalfa and other legumes may increase soil nitrogen enough to contribute to nitrate pollution of soil or ground water.

- # *Alteration of host range*. Alteration of host range can result in effects on legumes other than those that *R. meliloti* is known to infect. However, host range alteration appears unlikely for the submitted GEMs because no manipulations occurred in the loci important to host range specificity.

- # *Effects on nonlegumes.* Because naturally occurring rhizobia have no effect on nonlegumes, including those grown in rotation with alfalfa, effects on nonlegumes appear unlikely. In addition, information about the constructs gives no reason to suspect such effects.

- # *Effects on sweet clover and fenugreek.* Increased growth of the sweet clover (*Melilotus*) when it occurs as a weed in another crop could adversely affect the agroecosystem by decreasing the quality of the planted crop or by increasing production of coumarin, a secondary metabolite found in the sweet clover plant that is hazardous to livestock. Decreased growth of fenugreek or of sweet clover (when grown as a crop) also could adversely affect certain agroecosystems. The greenhouse and field data on alfalfa yield would not be predictive of the effects of rhizobia on these other legumes.

- # *Spread of antibiotic resistance.* Large-scale releases offer a greater opportunity for transfer of these resistances to bacterial pathogens of humans and animals.

Comments on Analysis: Characterization of Ecological Effects

Strengths of the case study include:

- ! *The body of knowledge on the effects of previous uses of rhizobia (rhizobial inoculation has been practiced for almost a century) and the well-characterized strains in the case study compensate, in part, for the weakness of monitoring effects.*

Comments on Analysis: Characterization of Ecological Effects (continued)

Limitations include:

- *Neither the yield data from poorly designed and implemented greenhouse studies nor the highly variable data from the field tests themselves could reliably comment on the efficacy of the introduced genetically engineered rhizobia.*

- *Table 2-2 in the study lists 12 assessment endpoints and the sources of information used to evaluate them, but only alfalfa growth was addressed in the study. Off-site migration was considered, and the field test itself contributed data with regard to the movement of rhizobia off-site.*

2.3.5. Risk Characterization

2.3.5.1. Risk Estimation

The risk of conducting the small-scale field test was considered low. The field test would collect data on alfalfa yield and microorganism fate. Decreased alfalfa yields, increased competitiveness, or movement off-site could have triggered termination of the field test.

The study did not evaluate several assessment endpoints because of the small likelihood of off-site dispersal. Both the characteristics of the field site and the test protocol supported this position (see section 2.3.3.) The field site's low slope minimizes surface water runoff, and the site contains no wells. In addition, the test protocol also limited movement off-site through (1) the in-furrow spraying technique for rhizobial application, (2) on-site decontamination of equipment and disposal of plant material, and (3) growth of rye grass and uninoculated alfalfa borders around test plots. Further, monitoring of soil and water evaluated off-site movement, while the test protocol also established emergency termination procedures in the event that significant spread appeared likely. Data collected during the small-scale field test confirmed the prediction that only limited off-site movement of rhizobia would occur. Appendix C presents the results of the aerial, lateral, and vertical dispersion studies.

Even if dispersal had occurred, the numbers of microorganisms required for legume infection may have precluded effective nodulation of other legumes near the site. The PMN submission suggests 10^3 rhizobial cells/seed for agricultural application. Others have noted infection concentrations of 100 to 1,000 rhizobial cells/g soil for effective nodulation of legumes (van Elsas et al., 1990). Consequently, the assessment did not address large-scale effects such as effects on the nitrogen cycle and the spread of clinically important antibiotic resistances. To assess enhanced growth of weedy legumes, the study examined the 14-acre site for sweet clover and weedy *Medicago* species (as noted in section 2.3.3). The study did not assess exposure to the legume fenugreek because this crop plant grows only in certain portions of the United States.

Appendix B presents the data on the competitiveness and survival of the rhizobial strains, as measured by nodule occupancy and persistence in the rhizosphere. The low viability of some of the inoculant strains (appendix E) affects the data in appendices B and C. As predicted from the greenhouse data, the rhizobial strains became established and survived well in the rhizosphere. Nodule occupancy tests demonstrated that the inoculant strains were fairly competitive compared with indigenous rhizobial populations. The recombinant and naturally occurring strains showed no significant differences in survival or competitiveness.

2.3.5.2. Uncertainty

Both effects and fate data and information in the PMN had elements of uncertainty. For the greenhouse yield data, uncertainty resulted from the protocol, the alfalfa cultivar relative to the field trials, and the extrapolation to field results. The alfalfa yield in the field may not have reflected the ability of the rhizobia to increase alfalfa growth because the test did not measure total nitrogen in the field soil, and high levels of nitrogen can inhibit nodulation by rhizobia. Heavy weed and leaf hopper infestations also may have confounded the alfalfa yield data.

Uncertainty also exists regarding the effects on weedy legumes and other crop legumes in the cross-inoculation group for *R. meliloti*. For the GEMs undergoing field testing, no data existed that would have indicated their competitive ability to nodulate alfalfa relative to native rhizobia.

Fate data and information in the PMN also had elements of uncertainty associated with them. Extrapolation from pure laboratory culture and greenhouse studies to the field is questionable. How well the monitoring techniques could distinguish the released rhizobia from each other and from the indigenous rhizobia is also uncertain.

2.3.5.3. Risk Description

After completion of the field test, the risk assessment indicates that the likelihood of adverse effects occurring either in the field or beyond the field border is considered low because of limited dispersal from the site, site termination procedures, the number of rhizobia needed to infect alfalfa plants, competition from native rhizobia, and the natural decline in cell populations expected in the absence of further alfalfa planting. Other effects noted for large-scale release of rhizobia will be addressed should large-scale releases become likely (see section 2.3.3).

Comments on Risk Characterization

Strengths of the case study include:

- *The case study characterized as low the risk associated with limited release of genetically engineered rhizobia into a small-scale field site. This assessment was based on the generally held view that rhizobia are fairly innocuous bacteria, that the site would effect adequate containment of the released bacteria, and that the genetic construct would preclude transfer of the introduced* nif *genes as well as the antibiotic resistance markers to other strains. The reviewers generally were satisfied with that assessment.*

Comments on Risk Characterization (continued)

Limitations include:

- *The effects data on efficacy were lacking, and there was considerable uncertainty in monitoring data because of the limitations of the chosen methods. Some attempt should have been made to address these shortcomings.*

General reviewer comments:

- *The case study should have included a table that addressed the uncertainties introduced by the assumptions made. For example, the study assumed that the plate-counting technology used to estimate the spread of introduced strains can distinguish between the introduced strains and indigenous R. meliloti. Similarly, the study should either give a literature citation or acknowledge the following as an assumption: an infective dose of 10^3 rhizobial cells/seed establishes a safe level of escaped rhizobia at less than 10^3/g soil.*
- *The case study might formulate action thresholds that would trigger the termination of the small-scale field test. These thresholds should consider the minimal infective dose and available data on persistence of the GEMs in the soil. For example, a test would be terminated when plate-counting on medium X detects more than 1,000 GEMs/g soil.*
- *As in all risk assessments, difficulty quantifying the hazard quotient leads assessors to argue for reduced exposure. Reviewers generally agreed that the risk assessors should attempt to bring quantification of the risk components of stressors to state of the art.*
- *The reviewers also generally agreed that proper measurement endpoints should make possible a meaningful characterization of risk in the restricted small-scale test.*

Comments on Risk Characterization (continued)

Authors' comments:

- *It is inappropriate to establish a level of safety for escaped rhizobia at 10^3 cells/g soil for several reasons. First, it is impossible to establish a safety level of a certain number of microorganisms that is below the detection limit for that microorganism. Second, it is not known exactly how many rhizobia are needed for a nodule formation. As discussed in section 2.3.5.1, according to the PMN submission, 10^3 cells/seed is the international standard inoculation rate for* R. meliloti. *This rate is supposed to ensure that the inoculant strain will be able to outcompete indigenous rhizobia. Another report in the literature suggested that 10^2 to 10^3 cells/g of soil are needed for effective nodulation of legumes (van Elsas et al., 1990); however, no data were supplied in this paper, and no reference was given for where these particular data could be obtained. Third, knowledge of the ecology of rhizobia indicate that rhizobial numbers are greatest in the rhizosphere of leguminous plants and may drop off several orders of magnitude in the bulk soil away from the plant. Rhizobia populations are known to persist in soils at low numbers for long periods of time, but will increase dramatically if the leguminous host plant is introduced into that soil. Consequently, it is inappropriate to establish any specific number as a safe level of escaped rhizobia in soil, even if one defines the portion of the soil that one is sampling, and even if one were to select a specific number that actually could be measured in this study.*
- *Knowledge of rhizobial ecology precludes the formation of "action thresholds" for rhizobia. It is inappropriate to put exact quantitative values on what level is safe and what level would trigger emergency termination of the small-scale field tests because of (1) a general lack of knowledge of exactly how many rhizobia are needed for infection, (2) the variability in population densities in the rhizosphere vs. soil at increasing distance away from the plant roots, and (3) the ability to stimulate rhizobial growth even after several years by planting the suitable leguminous host as discussed above.*
- *The reviewers again request that state-of-the-art methodology be used. As discussed in the case study and above, at the time this review was conducted (1988), antibiotic-selective media supplemented with the fluorescent antibody technique and the MPN growth pouch methods were deemed appropriate for these field tests. Great advances in methodology for detection of microorganisms in the environment over the past few years may allow for greater sensitivity in measurements for future studies.*

2.4. DISCUSSION BETWEEN RISK ASSESSOR AND RISK MANAGER

The 5(e) Consent Order (DCO 50-899004545) summarized how to conduct the field test and which data to collect. The Consent Order specified the following items:

- The field test will use EPA-approved protocols that will describe test objectives, field site, methods of transport of microbes to site, methods to limit dissemination, methods for detection and identification, descriptions of sampling procedures, and analysis of data.

- # The test will provide data on the following (with proper controls): nodule occupancy for all four recombinant microbes; alfalfa yield effects of all four PMN microorganisms; persistence in the rhizosphere with RMB7101 and RMB7103; vertical dissemination of RMB7101 and RMB7103; horizontal dissemination of RMB7101 and RMB7103; and aerial dissemination of RMB7101 and RMB7103 beyond the test plot during inoculation and termination.

- # The test will comply with applicable provisions of the Good Laboratory Practice Standards (40 CFR 792).

- # Microorganisms not used in the test will be disposed of in accordance with the NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958).

- # Reports on progress of the field test will be provided every 3 months.

- # The company will terminate the test if an event occurs indicating that the microorganisms have caused an adverse effect that EPA believes presents an unreasonable risk of injury to the environment.

2.5. RISK VERIFICATION

2.5.1. Persistence

The small-scale field tests verified the risk assessment conducted for this PMN submission. As expected from knowledge of rhizobial behavior and from greenhouse data, the recombinant rhizobia persist in the rhizosphere of alfalfa plants (see appendix B). The recombinant strains that the field trials investigated for persistence—strains RmSF38, RMB7101, and RMB7103—survived at rates of 10^5-10^6 cells/g dry root into the second year of the field study.

2.5.2. Competitiveness

As an indication of competitiveness of the recombinant rhizobial strains relative to unmodified strains, the study included nodule occupancy tests. Those conducted in the greenhouse used recombinant strains similar to the subject GEMs, while those subsequently conducted in the field used subject GEM strains. Neither set of occupancy tests indicated any significant difference in nodule occupancy for recombinant and parental strains (appendix C). However, in the strain competition trials, the recombinant strains appeared somewhat less competitive than the wild types. Interpretation of the data from this latter study proved difficult, however, because problems with culture viability prevented the desired ratio of 1:1 for the application rate of recombinant:parental strain (appendix E).

2.5.3. Dissemination From the Test Site

Information in the literature suggested that little off-site movement of rhizobia would occur during the test studies. The various dispersal studies conducted during the field trials confirmed this prediction (appendix C).

2.5.4. Effect on Alfalfa Yield During Field Test

Appendix E presents the alfalfa yield from the field studies and compares these with the greenhouse studies submitted as part of the PMN. This information is useful for validating both the risk assessment done by OPPT and this case study performed under the framework guidance. As predicted from the laboratory and the greenhouse studies, the construct analysis, and the literature, no adverse effects on alfalfa growth occurred with any of the rhizobial strains tested. Significant increases in yield also did not occur. Most importantly, no significant differences occurred between the use of the wild-type parental strains and the recombinant rhizobial strains.

2.6. KEY TERMS

biovar—A group of bacterial strains that can be distinguished by special biochemical or physiological properties that are consistent (but insufficient to justify a subspecies name for the group).

cassette—Structural and regulatory DNA sequences introduced into a GEM that allow the GEM to express a phenotypic trait of interest to the PMN submitter.

construct—1. (adj.) Information describing the DNA and genetic manipulations used to create the GEM. Such information covers the cassette, site of cassette insertion, use of vector DNA, intermediate recipients, and final recipients of cassette sequences. 2. (n.) The final genetic makeup of a GEM, including information noted for use of this term as an adjective.

cultivar—A group of individual plants that differ from others within the species due to certain consistent phenotypic traits (synonym: "variety").

vector—DNA sequences such as plasmids used to move the DNA of interest (usually cassette DNA) from one organism to another.

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APPENDIX A

MONITORING AND ENUMERATION TECHNIQUES FOR RHIZOBIA

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MONITORING AND ENUMERATION TECHNIQUES FOR RHIZOBIA

The monitoring studies used only three strains: RmSF38, a spontaneously streptomycin-resistant mutant of the parental strain RCR2011, and two recombinants, RMB7101 and RMB7103, both of which are derivatives of RCR2011.

Selective antibiotic media differentiate the parental from the recombinant and from the indigenous rhizobial strains. The basic medium proposed for enumeration of all rhizobial isolates, RDM (rhizobia defined medium), consisted of the following (g/L): potassium gluconate 5.0, K_2HPO_4 0.22, $MgSO_4 \cdot 7H_2O$ 0.1, sodium glutamate 1.1, 1,000X trace elements, 1,000X vitamin stock, and agar. The parental strain RCR2011 was intrinsically resistant to kanamycin and cinoxacin at 10 μg/mL and 100 μg/mL, respectively. Medium A, proposed for enumeration of RmSF38, consisted of RDM supplemented with kanamycin (10 μg/mL), cinoxacin (100 μg/mL), and streptomycin (200 μg/mL) as well as the antifungal agents cycloheximide and nystatin, both at the rate of 75 μg/mL. Medium B, for enumeration of the recombinant strains RMB7101 and RMB7103, was identical to Medium A except for addition of another antibiotic, spectinomycin (100 μg/mL). Spectinomycin was needed because both streptomycin and spectinomycin resistances were carried on the Ω fragment that was inserted to make the recombinant strains.

Recovery studies revealed that 51 to 90 percent of added rhizobia were recovered from the Sun Prairie soil 1 hour after addition to the soil. The PMN contained data from preliminary laboratory studies indicating that indigenous rhizobia intrinsically resistant to the same antibiotics as the GEMs occurred in low numbers and did not increase greatly in the presence of plant roots.

For the fluorescent antibody technique (and for future measurements during the field tests), the study selected 20 colonies from each antibiotic plate to determine the percentage of colonies formed on that plate by the inoculant strain versus the indigenous rhizobial populations. Multiplying this conversion factor by the total number of colonies on the plates corrected for the inoculants and eliminated the indigenous rhizobia.

Dr. E.L. Schmidt at the University of Minnesota prepared the immunofluorescent antibody to the parental *R. meliloti* strain RCR2011 using antiserum collected from the first production bleed of an immunized New Zealand white rabbit. The fluorescent antibody was a conjugate of the IgG fraction of the antiserum to the fluorescent dye, fluorescein. Dr. Schmidt's laboratory titered the fluorescent antibody to determine the highest antibody dilution that provided an acceptable homologous cross-reaction against strain RCR2011. A 1:1 dilution of the antibody suspension in glycerol was diluted 1:2, 1:4, 1:8, and 1:16 in filtered saline, and each dilution was applied to microscope slides containing rhizobial smears. Cross-reactivity was rated as (-) = no reactivity, tr = trace, and from (1+) to (4+) indicating very weak to strong cross-reactivity. The 1:16 dilution exhibited cross-reactivity of 4+ with RCR2011 derivatives but no cross-reactivity with the other rhizobial parental strains, their derivatives, or indigenous rhizobial populations. Consequently, this dilution was used for all further work. Laboratory tests conducted prior to the field tests indicated an MDL of 5×10^3 cells/g dry soil with this supplemental fluorescent antibody technique.

Colony morphology also distinguished between the RCR2011 derivatives and the indigenous populations. The indigenous rhizobia produced mucoid colonies, whereas the RCR2011 derivatives were always nonmucoid.

Although other aspects of population dynamics studies used all strains, dispersal monitoring used only the RCR2011 derivatives. Neither the PC parent or derivatives nor the UC445 parent or derivatives had good enough antigenic properties to produce a fluorescent antibody usable for detection. In addition, the highly mucoid PC strains were indistinguishable from the indigenous population. The RCR2011 strain and its derivatives served as an appropriate model for microbial dispersal, making it unnecessary to investigate all strains.

APPENDIX B

PERSISTENCE IN THE RHIZOSPHERE AND NODULE OCCUPANCY

Persistence in the Rhizosphere

The 2-year study followed the establishment and persistence of three strains—a wild-type strain, RmSF38, and two recombinant strains, RMB7101 and RMB7103—by means of selective media plating. All three strains were established in the rhizosphere and remained stable through the 1989 growing season at levels of approximately 10^6 in the inner rhizosphere and 10^5 to 10^6 cells/g dry root in the outer rhizosphere. The first sampling in April 1990 revealed rhizobial numbers in the inner and outer rhizosphere similar to the levels for the last sampling of the 1989 season, indicating that the rhizobial strains either overwintered at these levels or recovered after thawing in the spring. Although all three strains persisted in the rhizosphere through day 376, the levels of the two recombinant strains were approximately tenfold lower than the level of indigenous rhizobia. In summary, both the wild-type and the two recombinant strains became established in the rhizosphere and persisted into year 2, in general showing no population differences.

Nodule Occupancy

The strain comparison trial on October 3, 1989, entailed nodule occupancy studies. The study measured the length of the root systems for 12 plants, with the root system being divided into four sections: crown, top middle, bottom middle, and distal. A maximum of 24 nodules from each section was screened for the presence of the inoculant. Unfortunately, the parental strain PC and the indigenous rhizobia were indistinguishable. However, the other parental strains and the recombinants could be identified. The data indicated that nodule occupancy ranged from 39 to 70 percent for the inoculated rhizobial strains, the remaining nodules being occupied by the indigenous rhizobia. The percent nodule occupancy by the inoculant decreased with increased distance from the crown in all cases. No significant differences occurred between the wild-type and recombinant strains. Plants collected in the second year, 15 days prior to the second harvest, showed a decline in percent nodule occupancy for all inoculated treatments.

In the strain competition trial, parental and recombinant strains were inoculated together. Nodule occupancy data showed that recombinant strains appeared somewhat less competitive than the wild types. Because problems with culture viability prevented the desired inoculation ratio of 1:1, interpreting these data is difficult (appendix D).

APPENDIX C

RHIZOBIAL DISPERSION AND MIGRATION

Aerial Dispersal

Selective agar plates were mounted on posts located in all four compass directions at various distances—4, 9, 50, 100, 200, and up to 500 feet—from the perimeter of the test plots on days 0, 1, 2, 3, 4, and 6 after initiation of the strain comparison trial. Additional plates were placed between the four compass points. No colonies appeared on the vast majority of plates regardless of compass direction or distance. A total of 13 colonies appeared on Selective Medium A over a cumulative exposure of 6 hours on day 0 for all compass directions and distances even though a moderate wind blew on the day of application. Later samplings were for 2-hour exposures only. On day 6, the number of colonies on Medium A from the west compass direction (the direction with the highest counts) had dropped from 13 at the 4-foot distance to one colony at both the 100- and 200-foot distances. Overall, little aerial dispersion of the PMN microorganisms occurred. Likewise, aerial dispersion measurements taken at termination, when the fields were being plowed, resulted in no detectable dispersal of inoculant from the test site.

Vertical Migration

Movement of the recombinant rhizobia downward through the soil profile past the rhizosphere was measured by plating out soil obtained with a soil-coring device. Twelve-inch cores were taken from control and treated plots in an outside row, immediately adjacent to a plant stalk. The top 2 and bottom 2 inches of the soil core were homogenized and subsampled for the presence of added rhizobia.

Vertical monitoring used the plant MPN technique for enumeration at various time points up to 312 days. Throughout the season, cell numbers ranged from 7 to >138 cells/g dry soil in the top 2 inches and from 3 to >524 cells/g dry soil in the 10- to 12-inch depth. Rhizobial inoculants also occurred at a depth of 22 to 24 inches. Overall, only minimal movement occurred beyond the root zone. No differences occurred in the vertical movement of the recombinant strains versus the wild-type strain.

Horizontal Dispersion

The study monitored horizontal movement through the soil by sampling the top 2 inches of the soil surface at a distance of 6 inches away from the edge of the plots in all four compass directions on days 0, 11, and 34. Samples were examined for the presence of three strains: RmSF38 and two recombinants, RMB7101 and RMB7103. Using selective media supplemented with the fluorescent antibody method, samples contained no detectable inoculants. With the more sensitive MPN enumeration technique, counts ranged from 0 to 57 cells/g dry soil. Consequently, all subsequent analyses used the MPN technique. Up through day 123, cell counts never exceeded 250 cells/g dry soil, and nearly all counts dropped to 0 by day 159. These results indicate minimal horizontal movement of the rhizobial inoculants throughout the study and no differences in the behavior of the recombinant strains versus the wild type.

APPENDIX D

STRAIN COMPARISON AND COMPETITION TESTS

APPENDIX D
STRAIN COMPARISON AND COMPETITION TESTS

The strain comparison test used four recombinant strains and a single alfalfa variety. The total area for the strain comparison trial was approximately 0.65 acre, with 0.07 acre treated with recombinant rhizobia. The proposed design consisted of 13 treatments set up as a complete randomized block design with six replicates. Each treatment occupied a plot measuring 5 × 25 feet. A 5-foot wide buffer strip of ryegrass separated plots from each other. A 5-foot wide border of uninoculated alfalfa surrounded the experimental area. Alfalfa seeds were planted with a cone planter in rows 6 inches apart and sown to a depth of approximately 0.25 to 0.5 inches. A carbon dioxide-propelled bicycle sprayer, calibrated to deliver 10 mL/linear foot, sprayed 3.0 L of suspensions of each rhizobial strain on the alfalfa seeds in the open furrows. The application rate was approximately 10^5 bacteria per seed. This rate corresponded to 2.3×10^{11} seeds per plot, for a total of 5.52×10^{12} recombinant *R. meliloti* cells. Immediately following spraying of the rhizobia, garden rakes were used to cover the furrows with soil.

The strain competition experiments took place on a 0.09-acre portion of the same field (48 × 78 feet). The proposed design consisted of 22 treatments set up as a randomized complete block design with four replications. Each treatment consisted of one row, 6 feet long, with seeds spaced every 0.5 to 1.0 inch. Rows were 3 feet apart. Because of the experiments' short duration (8 weeks), the ryegrass borders were omitted. As in the strain comparison trial, a 5-foot wide border of uninoculated alfalfa surrounded the entire test area. A hand-held spray bottle sprayed 50 mL of rhizobial suspension into each 6-foot furrow row. At an inoculum rate of approximately 10^5 bacteria per seed, each treatment had a total of 1.2×10^{10} rhizobial cells. This corresponded to a total application of 5.52×10^{11} recombinant rhizobial cells. Then the furrows were covered with soil.

Although the test design called for applying *R. meliloti* strains at a rate of 10^5 cells per seed to obtain 100 times the international minimum standard for alfalfa of 10^3, the actual viable counts applied in the field were significantly lower. For results of viability studies, see appendix E.

APPENDIX E

RHIZOBIAL CULTURE VIABILITY

APPENDIX E
RHIZOBIAL CULTURE VIABILITY

To determine the actual application rate of the *R. meliloti* strains sprayed on the seeds, aliquots of the rhizobial suspensions were plated onto selective media to measure culture viability. The following table summarizes the results.

	% of Anticipated Viable Cells	
Applied Strain	Strain Comparison	Strain Competition
RCR2011 (parent)	97	60
RMB7101 (RCR2011 parent + Ω)	113	10
RMB7103 (RCR2011 parent + Ω + *nif*)	89	20
PC (parent)	49	20
RMB7201 (PC parent + Ω)	43	40
UC445 (parent)	77	5
RMB7401 (UC445 parent + Ω)	50	5

Note that in some cases the numbers obtained are much lower than the number of viable cells intended for application. This situation is particularly true for the strain competition trial. Consequently, the strain competition trials often did not have the desired 1:1 ratios. The ratios of parent:recombinant for recombinants RMB7101, RMB7201, RMB7401, and RMB7103 were 1:0.85, 1:1.1, 0.34:1, and 1:0.43, respectively.

APPENDIX F

ALFALFA YIELDS IN THE FIELD TESTS

Table F1. Alfalfa Yields in Year One—First Cutting

Treatment	Alfalfa Yield (kg/ha)
RCR2011	3,295
RMB7101 (RCR2011 parent + Ω)	3,362
RMB7103 (RCR2011 parent + Ω + *nif*)	4,707
PC	3,766
RMB7201 (PC parent + Ω)	3,071
UC445	3,295
RMB7401 (UC445 parent + Ω)	3,676

The naturally occurring and recombinant strains tested gave no significant differences in the dry weight yield of alfalfa at the first cutting (coefficient of variance [C.V.] 42.45 percent). This result may have occurred, in part, because of the variable stand of alfalfa often observed the first year after planting. The test plots suffered heavy weed infestation (no preplant herbicide was used), and the alfalfa plants also suffered stunting and chlorosis as the result of a heavy leaf hopper infestation in early July. To allow for spraying for leaf hoppers, the first cutting occurred earlier rather than the normal 10 percent bloom standard.

The table below presents the dry weight yields of alfalfa for the second cutting, which occurred 44 days after the first cutting.

Table F2. Alfalfa Yields in Year One—Second Cutting

Treatment	Alfalfa Yield (kg/ha)
RCR2011	3,295
RMB7101	3,049
RMB7103	3,362
PC	3,004
RMB7201	2,892
UC445	3,049
RMB7401	3,049

Again, the naturally occurring and the recombinant strains resulted in no significant differences in the dry weight yield of alfalfa (C.V. 11.74 percent). The test plots again showed heavy weed infestation.

Alfalfa was harvested twice in the second year of the field tests, once on June 6 and 7 and again on July 24. The table below presents data for dry weight yield.

Table F3. Alfalfa Yields in Year Two—First and Second Cuttings

Treatment	Alfalfa Yield (kg/ha) 1st Cutting	2nd Cutting
RCR2011	4,304	6,052
RMB7101	4,102	6,232
RMB7103	4,416	6,590
PC	4,281	6,254
RMB7201	4,506	6,590
UC445 4,304	6,209	
RMB7401	4,438	6,276
C.V. (%)	11.50	6.95

The second year of the strain comparison test showed no significant differences in alfalfa dry weight yield with wild-type and recombinant *R. meliloti* strains. The second year's alfalfa growth lacked much of the variation seen in the first year. Consistent trends, however, were not evident.

The field data showed no conclusive trends toward either increased or decreased growth of alfalfa as compared with the parent strains. Therefore, the field tests indicate that the recombinant rhizobia posed little risk of decreasing alfalfa yields. The greenhouse data, although faulty, also indicated little potential for decreased growth of alfalfa from the GEMs.

SECTION THREE

ECOLOGICAL RISK ASSESSMENT CASE STUDY:

EFFECTS OF RADIONUCLIDES IN THE COLUMBIA RIVER SYSTEM—A HISTORICAL ASSESSMENT

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LIST OF ACRONYMS

BCF	bioconcentration factor
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act of 1980
DOE	Department of Energy
HQ	hazard quotient
NRDA	Natural Resource Damage Assessment
PNL	Pacific Northwest Laboratory
RM	River Mile
USGS	United States Geological Survey

ABSTRACT

In 1943, nuclear production activities began at the U.S. Department of Energy's (DOE) Hanford site in south-central Washington State. These activities continued for many years. During this time, the site discharged radioactive effluents into the Columbia River, which runs through the northern portion of the site and borders it on the east (the Hanford Reach). The DOE requested the Pacific Northwest Laboratory (PNL) to conduct an ecological risk assessment to determine whether the ecological risk assessment framework (EPA, 1992) used for hazardous chemicals is applicable to radionuclides as stressors. PNL conducted this ecological risk assessment using historical Hanford site monitoring data, which had been collected to characterize human dose. The data characterized exposure by measuring radioactivity in water, sediments, and biota. The data used in the current investigation were collected during 1963-1964, a period of peak production of nuclear material. During this time, the maximum number of eight reactors were operational.

PNL employed two approaches in assessing ecological risk to Columbia River organisms. The first approach used environmental exposure data (water concentrations for radionuclides) to calculate dose to a variety of aquatic organisms, including the most sensitive receptors (fish). The second approach made use of measured tissue concentrations of selected aquatic organisms to calculate organism internal dose.

PNL used dose to assess potential toxic effects and assess regulatory compliance. Risk characterization was developed by comparing dose levels in fish and other organisms found in the Columbia River to known effect concentrations through a hazard quotient for acute dose and possible developmental effects. The assessment endpoint was protection of fishes in the Columbia River, and the measurement endpoint was increases in mortality and sublethal effects. One of the most sensitive ecological receptors was the early developmental stage of chinook salmon.

The major conclusions of the study are:

The ecological risk assessment paradigm is applicable to radionuclides as well as to hazardous chemicals, as evidenced from the exposure, effect, and risk characterization.

The most sensitive life stage of fish (i.e., salmon embryo) did not appear to be at risk from radionuclide exposure in sediments or water.

During peak production at Hanford, releases of radionuclides did not result in any measurable risk to the Columbia River ecosystem, as evidenced by indicator species and regulatory benchmarks.

Dose rates to Columbia River animals during the study period did not exceed the DOE standard of 1 rad/d per DOE Order 5400.5 (DOE, 1989). Based on the computer code CRITR2, only crayfish and a plant-eating duck received a dose rate exceeding 1 rad/d. However, this risk assessment did not include ducks, and the actual calculation of dose to crayfish from whole organism counts gave values considerably less than both the modeled dose and 1 rad/d.

3.1. RISK ASSESSMENT APPROACH

The ecological risk assessment follows the sequence of the U.S. Environmental Protection Agency's Framework for Ecological Risk Assessment (EPA, 1992). This arrangement includes problem formulation, analysis, and risk characterization, respectively (figure 3-1).

Exposure of aquatic organisms to radioactivity can elicit a toxic response depending on the organism, level of dose, type of radionuclide, and habitat requirements of the exposed organism. In this study, the assessment endpoint was defined as the maintenance of important recreational and commercial fish populations in the Columbia River. The measurement endpoint from radioactive dose was toxicological response. This assessment did not consider elemental chemical toxicity of each radionuclide.

The major ecological components are benthic macroinvertebrates, zooplankton, phytoplankton, and fish of the Columbia River. Fish species in the Columbia River are important commercial, recreational, cultural, and regional assets.

Data analysis included exposure and effects characterization. Exposure characterization consisted of an assessment of radioactivity at several river stations downstream from the Hanford site. Measured river activity was used to calculate ionizing radiation dose from water to selected organisms using bioaccumulation factors and computer modeling. A second and more direct means of estimating dose to aquatic organisms used measured fish tissue concentrations. Available sampling data included sediments, water, and biota.

Characterization of effects to aquatic organisms entailed using available toxicity data and regulatory standards. The characterization was conducted at the individual level, qualitatively interpreted, and applied to the population level of ecological organization. Risk characterization was based on a hazard quotient (HQ), defined as the ratio of radionuclide organism dose (exposure or tissue value) to benchmark dose values.

3.2. STATUTORY AND REGULATORY BACKGROUND

Although federal regulations do not require quantitative ecological risk assessments, they can be used effectively to support regulatory requirements under nearly all of the major federal environmental statutes (e.g., the Comprehensive Environmental Response, Compensation, and Liability Act, CERCLA). Other potential applications include supporting compliance with federal Executive Orders and with policy directives of various government agencies (e.g., DOE Orders).

A number of federal statutes have promulgated risk-based and technology-based standards for the protection of ecological resources (e.g., water quality criteria under the Clean Water Act). However, only one standard has been published for the protection of ecological resources from exposure to radioactive materials. DOE Order 5400.5 (DOE, 1989) stipulates that the interim dose limit for native aquatic animal organisms "shall not exceed 1 rad per day from exposure to the radioactive material in liquid wastes discharged to natural waterways."

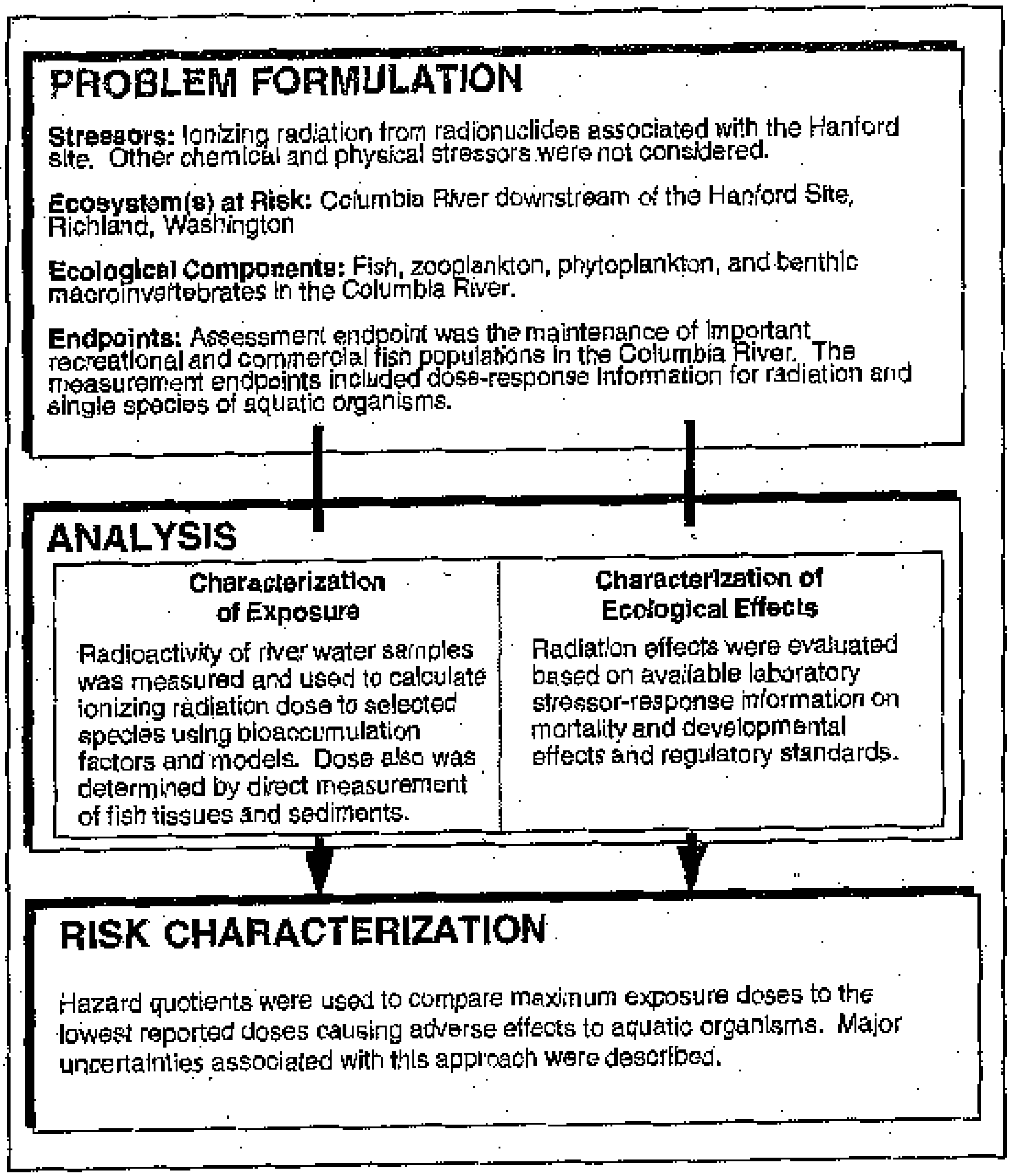

Figure 3-1. Structure of assessment for effects of radionuclides

3.3. CASE STUDY DESCRIPTION

3.3.1. Background Information and Objective

It is generally assumed that human health risk standards for radionuclides protect wildlife sufficiently. However, under some circumstances the risk to wildlife from radionuclides may need to be considered, such as managing risks, developing cleanup strategies, and identifying injury under the Natural Resource Damage Assessment (NRDA) process. The objective of this case study is to evaluate the applicability of the ecological risk assessment paradigm for radionuclides as stressors in the Columbia River.

The Hanford site, an area of slightly more than 1,400 km^2 (560 mi^2), straddles the Columbia River just north of Richland, Washington. Three northwest-southeast-trending basalt ridges cross this broad, relatively level gravel plain. The semiarid climate supports various communities of shrubs—steppe and grassland.

The Columbia River extends 1,954 km (1,214 mi) from its origin in Columbia Lake in British Columbia to its mouth at Astoria, Oregon, making it the fourth-longest river in North America. Typical flow rates of the Columbia River at Priest Rapids Dam range from 2,800 to 3,400 cubic meters per second (cms), or 99,000 to 122,000 cubic feet per second (cfs) (Woodruff et al., 1991).

The Columbia River has eight primary uses:

1. River navigation through navigation locks from the Pacific Ocean to the Port of Benton in Richland.
2. Agricultural purposes, primarily irrigation. Approximately 6 percent of the Columbia Basin's water is diverted for agricultural use.
3. Nonagricultural irrigation.
4. Electric power generation, provided by the system of 11 dams along the Columbia River in the United States.
5. Flood control, also provided by the dams.
6. Fish and wildlife habitat, especially for anadromous salmon. The Hanford Reach comprises the last major salmon and steelhead spawning area within the Columbia River proper. The Columbia River also supports the vast majority of mesic terrestrial habitat in the semiarid Hanford Reach.
7. Water supplies to numerous municipalities and industries.
8. Recreational use.

The Hanford Reach of the Columbia River runs from Priest Rapids Dam to just north of the City of Richland and flows past the reactor areas of the Hanford site (figure 3-2). The average annual flow of the Columbia River in the Hanford Reach, based on 65 years of record, is about 3,400 cms (120,100 cfs) (DOE, 1988). Flows in the Hanford Reach vary widely, not only because of the annual flood flow but also because of daily regulation by the upstream power-producing Priest Rapids Dam. Flow rates during the late summer, fall, and winter may vary from a low of 1,100 cms (36,000 cfs) to as much as 4,800 cms (160,000 cfs) each day. During the spring runoff, peak flow rates from 4,800 to 20,000 cms (160,000 to 650,000 cfs) can occur.

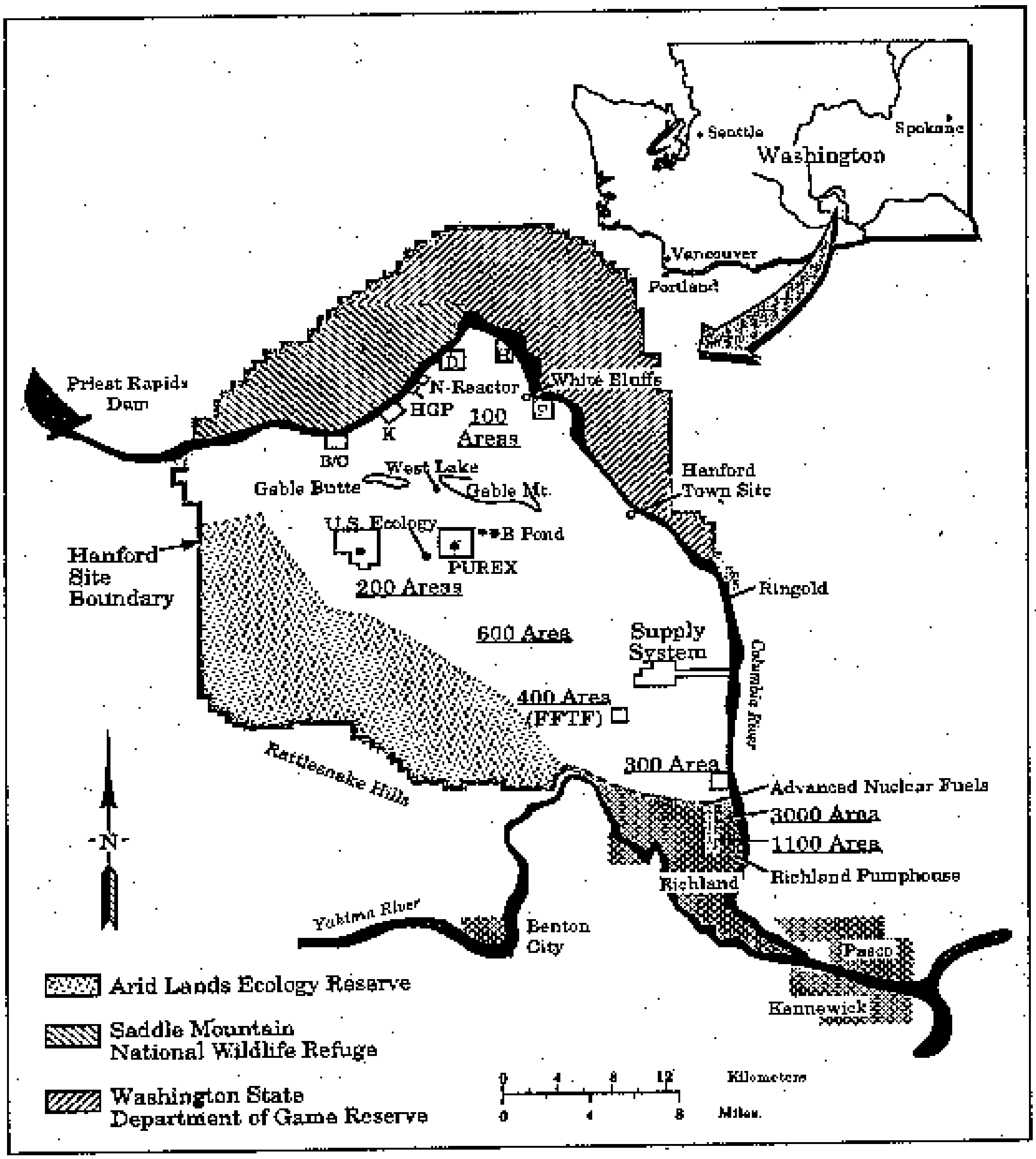

Figure 3-2. Location of the Hanford Reach of the Columbia River

The Washington State Department of Ecology classifies the Columbia River water quality as Class A (excellent) between Grand Coulee Dam and the mouth of the Columbia River (DOE, 1988). Table 3-1 shows water quality data between Priest Rapids Dam and Pasco, Washington, for the years 1957-1973. The dominant physical feature of the Columbia River through the Hanford Reach is the high flow rate, which is subject to large, diurnal water-level fluctuations that change the shoreline configuration and expose gravel substrate and periphyton to alternate periods of wetting and drying. The Reach has a low level of suspended sediment, 1 to 7 mg/L.

The river-bottom sediments from Priest Rapids Dam to several kilometers below the confluence of the Snake and Columbia Rivers are primarily mixed sands and gravels with some cobbles (maximum diameter ≈ 20 cm). Coarser sediments predominate from Priest Rapids Dam through the reactor areas (DOE, 1988). The streambed near Richland consists of sand in deep channels and a mixture of sand, silt, and some clay in shallow areas (DOE, 1988). Most of the Hanford-produced cationic radionuclides are associated with suspended particulates and subsequent fine sediments (Beasly and Jennings, 1984).

Because of the many dams on the Columbia River, the only free-flowing U.S. section occurs between Priest Rapids Dam (River Mile [RM] 397) and McNary Reservoir (RM 351). The Priest Rapids Dam immediately upstream from the Hanford site regulates flow. No significant tributaries enter the stream in this section, which lies mostly within the Hanford site.

The main channel of the Hanford Reach is braided around the island reaches and submerged rock ledges and gravel bars, causing repeated pooling and channeling. The riverbed material is mobile and dependent on river velocities; it typically is composed of sand, gravel, and rocks up to 20 cm (8 in) in diameter. Small fractions of silts and clays are associated with the sands in areas of low-velocity deposition.

3.3.2. Problem Formulation

3.3.2.1. Stressors

The release of radionuclides from Hanford operations is one of several possible stressors to the ecosystems of the Columbia River. Other possible stressors include thermal discharges from Hanford reactors; varying river levels because of dams; the physical barrier to fish migration from the dams; and heavy agricultural, commercial, and recreational activities along the river. However, this assessment concerns only radionuclides as stressors of concern.

The cooling effluents of Hanford reactors contain over 60 radionuclides. Becker (1990) has reported that during the period of maximum reactor production (mid-1960s), the Hanford site discharged over 300,000 curies per year to the river. Radioactive decay influenced the relative abundance of different radionuclides in the river (Becker, 1990). In fact, many of the radionuclides discharged by the Hanford site have a short half-life and were not detected in the effluent discharge. Others could not be detected in the river after dilution. Becker (1990)

Table 3-1. Summary of Water Quality Data, 1957-1973 (DOE, 1988)

Location/ Statistic	DO[a] (mg/L)	Temperature (°C)	Coliform (MPN[a]/100 mL)	pH	Color (PT-CO[a] units)	Hardness (mg/L)	Turbidity (JTU[a])	PO_4-P (mg/L)	Ortho NO_3-N (mg/L)
Below Priest Rapids (River Mile 395)									
Minimum	9.5	1.8	0	6.5	0	55	0	0.01	0.02
Mean	11.9	11.4	131	7.7	5	69	3	0.08	0.10
Maximum	15.9	19.2	2,000	8.5	33	81	29	0.15	1.50
Pasco (River Mile 330)									
Minimum	6.8	3.0	1	6.8	0	40	0	0.01	0.05
Mean	10.8	12.2	182	8.1	8	73	15	0.10	0.19
Maximum	14.3	22.0	4,800	8.6	68	90	140	0.02	0.37

[a]DO = Dissolved oxygen.
JTU = Jackson turbidity units.
MPN = Most probable number.
PT-CO = Platinum-cobalt.

identified three radionuclides as being of concern because of their potential biological significance: phosphorus-32, chromium-51, and zinc-65. Together they account for over 90 percent of potential radiological dose to aquatic organisms. All are nuclear activation products that are activated as Columbia River water cools the reactor core. The potential for some radionuclides to bioaccumulate in aquatic food webs causes concern with respect to both the human exposure pathways and potential ecosystem effects.

Among radionuclides, phosphorus-32 and zinc-65 are potential stressors because of their biological importance and fate: they are essential elements for organism growth and are incorporated into the aquatic food web. One study conducted in the Hanford Reach from 1961 to 1968 noted a seasonal pattern of uptake by algae, with higher radioactivity in winter and lower in summer (Becker, 1990). This pattern reflects concentration and dilution phenomena from river flows.

Unlike phosphorus-32 and zinc-65, chromium-51 is not considered a major biological hazard. This radionuclide has a short half-life, low biological mobility (i.e., it has no known essential role in the physiology of organisms), and weak radiations. It does not accumulate to any extent in aquatic organisms and is transported with river-suspended particulate material with little dissolution (Becker, 1990). However, the risk assessment included it because it was a significant activation product.

The half-lives of the three radionuclides considered in the risk assessment are:

- Phosphorus-32: 14.2 days
- Chromium-51: 27.8 days
- Zinc-65: 245.0 days

Phosphorus-32 is a beta emitter (negatrons); chromium-51 emits gamma radiation and electrons; and zinc-65 is primarily a gamma emitter, but also emits positrons and electrons.

3.3.2.2. Biological Fate of Radionuclides

Phosphorus, including phosphorus-32, is a building block of various tissues and is a key element in many biochemical transformations, especially energy transduction (ATP, ADP, GTP, etc.). The element is comparatively scarce in the environment. Organisms can concentrate phosphorus, including phosphorus-32, to levels that greatly exceed the concentration in the ambient media. Phosphorus has a bioconcentration factor (BCF) of 24,000 for freshwater plants and 8,000 for freshwater animals (Becker, 1990).

Terrestrial plants take up little chromium-51 from soils, <0.5 percent (Becker, 1990). In aquatic systems, this element sorbs to particulate material and is transported along with it. Becker (1990) reported that in biological systems chromium-51 has an affinity for the blood of fish.

Organisms accumulate a measurable fraction of zinc-65. In aquatic systems, this radionuclide is transported through aquatic food webs. With chronic uptake, substantial tissue accumulation can occur. In the Pacific Ocean, Becker (1990) noted BCFs of up to 10^3 for algae and 10^5 for certain molluscs. The BCF for plankton in the Columbia River ranges from 300 to 19,000 (Cushing and Watson, 1966; Cushing, 1967a, b), with adsorption as the primary means of uptake. Because of its long half-life and biological mobility, zinc-65 can be transported through food webs.

3.3.2.3. Ecosystem Potentially at Risk

The Columbia River supports a diversity of aquatic and terrestrial wildlife. The major ecological components are benthic macroinvertebrates, zooplankton, phytoplankton, and fish. Although a detailed description of the wildlife exceeds the scope of this effort, appendix A lists the fish species and shows the generalized aquatic food web. This risk assessment focuses on the fish of the Columbia River because they are aquatic organisms sensitive to ionizing radiation and because the Columbia River supports a wide variety of fish, including several species that are commercial, recreational, and cultural assets of the region.

3.3.2.4. Endpoint Selection

Exposure of aquatic organisms to radioactivity can elicit a toxic response depending on the dose level, the length of exposure, the particular species, and the life stage at the time of exposure. The magnitude of the response is proportional to radiological dose. In this study, the assessment endpoint was the health and condition of local populations of selected fish species that were of commercial, recreational, and cultural interest.

The risk assessment evaluated multiple measurement endpoints. They included literature investigations of adverse effects on fish, such as acute mortality and sublethal and developmental effects. Dose from ionizing radiation was evaluated in the maximally exposed individual fish and fish in early developmental stages during the study period. Because no net increase occurred in the concentration of elements, the assessment considered only toxicity resulting from ionizing radiation, not toxicity resulting from chemical characteristics.

3.3.2.5. Conceptual Model

Radionuclides in the Columbia River are partitioned between river water, sediment, and the aquatic food web. Organisms become exposed through direct contact with river water, through contact or ingestion of contaminated sediments, or through food web incorporation of radionuclides.

Two organism exposure pathways exist for ionizing radiation. In the external exposure pathway, an organism receives a dose from its external environment, such as ionizing radiation from the water. If the energy of the radiation is high enough, it may penetrate the organism's external tissue. In the internal exposure pathway, an organism receives a dose of ionizing radiation as a result of uptake of a radionuclide. Consequently, exposure occurs to internal organs and tissues. The significance of each exposure pathway depends on the aquatic fate of the radionuclide, its concentration, the energy of its radiation, and also on the pathway of bioaccumulation.

The level of organism dose from either external or internal exposure depends on the length of time an organism spends in the Hanford Reach feeding and breeding habitats, the degree of interaction with the sediments (i.e., living on or in the sediments), the discharged levels of radionuclides, and the river flows. Potential dose to aquatic organisms equals the sum of the total ionizing radiation dose from multiple radionuclides.

Possible exposure scenarios include organisms living near or in reactor effluent discharges, at various locations downriver of Hanford, and on or in contaminated sediments. A resident fish, such as whitefish, can spend its entire life in the Hanford Reach. The adult chinook salmon, on the other hand, is present only during selected periods of the year.

Generally, higher-level organisms such as fish have greater sensitivity to ionizing radiation than lower-level organisms such as algae and invertebrates (Frank, 1973). Consequently, fish can serve as indicators or benchmarks of the health of fish populations and the ecosystem. For fish, sensitivity varies with developmental stage, (i.e., adult fish being less sensitive then juveniles), amount of time required for various developmental stages, and number of fertilized eggs produced (Whicker and Shultz, 1982). Species fecundity factors into extrapolating individual organism effects to a population. For example, species with high fecundity rates most likely will not experience adverse effects to the same degree as species with low fecundity rates. In addition, the exposure of organisms to low-level ionizing radiation can promote injury repair mechanisms.

For Hanford, most of the available monitoring data for radionuclides were for river water activity and tissue concentrations of selected species of fish, including mountain whitefish (*Prosopium* *williamsoni*). One of the most fished species in the Columbia River, mountain whitefish remains resident throughout the year, making it a useful biomonitor of radionuclide incorporation into the human food chain. The food chain accumulation of radionuclides by whitefish occurs in a three step process:

Water → Algae → Insects → Whitefish

Calculated dose to whitefish can be extrapolated to other fish species, such as adult chinook salmon that occur seasonally in the Hanford Reach of the Columbia River. In the risk assessment, whitefish served as an indicator or "generic" fish to develop a potential exposure/dose scenario. Where available, the risk assessment incorporated data for other fish species along with supportive or ecosystem descriptive data for phytoplankton, snails, and crayfish. Dose was estimated from exposure to measured radionuclides in the river to salmon embryos, identified as one of the most sensitive organisms to ionizing radiation.

Comments on Problem Formulation

Strengths of the case study include:

- *This case study was well written and well organized. It is an ideal case for the application of the EPA Risk Assessment Framework because discrete stressors are easily identified and measured and substantial data are available on their biological impacts. Assessment and measurement endpoints are identified and fit nicely into the risk assessment paradigm.*

Limitations include:

- *Because the Columbia River ecosystem has been affected by many other factors, radiation may have a relatively small impact on salmon. Therefore, although it may be valid to restrict the risk assessment to a single stressor that does not reflect the "real world" situation, other stressors on salmon should be identified.*
- *The authors should point out that data were developed for the specific case study, rather than for a full-ranging risk assessment that could consider other stressors. DOE and EPA need to know whether radionuclides are a major problem or risk to the ecosystem is negligible.*
- *The total biological community is not well characterized.*

3.3.3. Analysis: Characterization of Exposure

Making use of the 1963-1964 data for sediments, water, and biota, the exposure characterization employed two approaches to evaluate dose, which provided independent assessments of dose. The first approach evaluated river radioactivity at several stations downstream of the Hanford site. This approach then modeled organism dose using biological accumulation factors for several "generic" aquatic organisms from measured radionuclide water concentrations during the study period, 1963-1964. The second approach used measured radionuclide tissue concentrations to calculate dose to whitefish. Directly measured tissue activity has the advantage of considering all environmental pathways: water and food uptake, excretion, sediments, etc. However, this approach has the disadvantage of measuring selected radionuclides only in fish muscle tissue. As a result, the approach reflects the human pathway and places less emphasis on effects to the fish. For example, although organs and bones also accumulate radionuclides, they were not included in the dose calculation.

3.3.3.1. Sample Location

The initial exposure characterization was limited to the Richland Station (RM 344), although ultimately all available data from the Hanford Reach were reviewed and considered. The U.S. Geological Survey (USGS, 1966) indicated that the river is vertically and horizontally mixed at this point. This approach was used because of the potential for large spatial and temporal variability of radionuclide concentrations upstream. This variability resulted from the discharge of eight production reactors with individual production schedules. Once established, the relationship between exposure and potential effects can be applied to upstream locations.

3.3.3.2. Data Analysis

The risk assessment reviewed three data sets to characterize exposure: measured radionuclide river concentrations, measured sediment concentrations, and measured fish tissue concentrations. The data were collected during routine monitoring of radionuclide concentrations in the Columbia River system. River water was collected as composite, grab, or cumulative samples. The sampling scheme varied over the 2-year period (table 3-2). Figures 3-3 and 3-4 show the monthly water grab sample concentrations for selected radionuclides over the 2-year period. Water concentrations were generally highest during the winter and late fall and lowest in the spring and summer.

3.3.3.3. Exposure From Measured River Water Concentrations

Exposure concentrations were established by reviewing measured river activity data to determine the relationships among composite, grab, and continuous samples: that is, to see whether one form of sampling yielded consistently higher water concentrations than another. The results of this analysis showed that the highest river concentrations of radionuclides occurred in whole-water grab samples.

An upper-boundary exposure concentration was derived by using the maximum observed grab sample water concentration for the 2-year study period for each radionuclide shown in table 3-3. These concentrations were assumed to represent the maximum concentration for exposure of river organisms. If the effect characterization indicated a potential risk, then more typical exposure concentration scenarios could be developed.

The maximum sediment concentration measured for each radionuclide was used to calculate organism dose.

Table 3-2. Water Sampling Matrix (1963-1964) (Dirkes, 1992; Haushild et al., 1966; Nelson et al., 1964)

Station Location	Type	Frequency[a]											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1963													
Richland	Grab		x[b]			x		x	x	x	x	x	x
Hanford	Grab	x	x	x	x	x	x	x	x		x		
1964													
Richland	Grab	x	x	x	x	x	x	x	x	x	x	x	x
	Cum	o	o	o	o	o	o	o	o	o	o	o	o
	Comp						*	*	*	*	*	*	*
Hanford	Grab	*	*	*	*	*	*	*	*				
	Comp	o	o	o	o	o	o	o	o				
	Cum	o	o	o	o	o	o	o	o				

[a]Totals for 1963: Grab--159.
Totals for 1964: Grab--149.
Comp--194.
Cum --300.

[b]Legend: x--every 2 weeks.
o--weekly.
*--monthly.

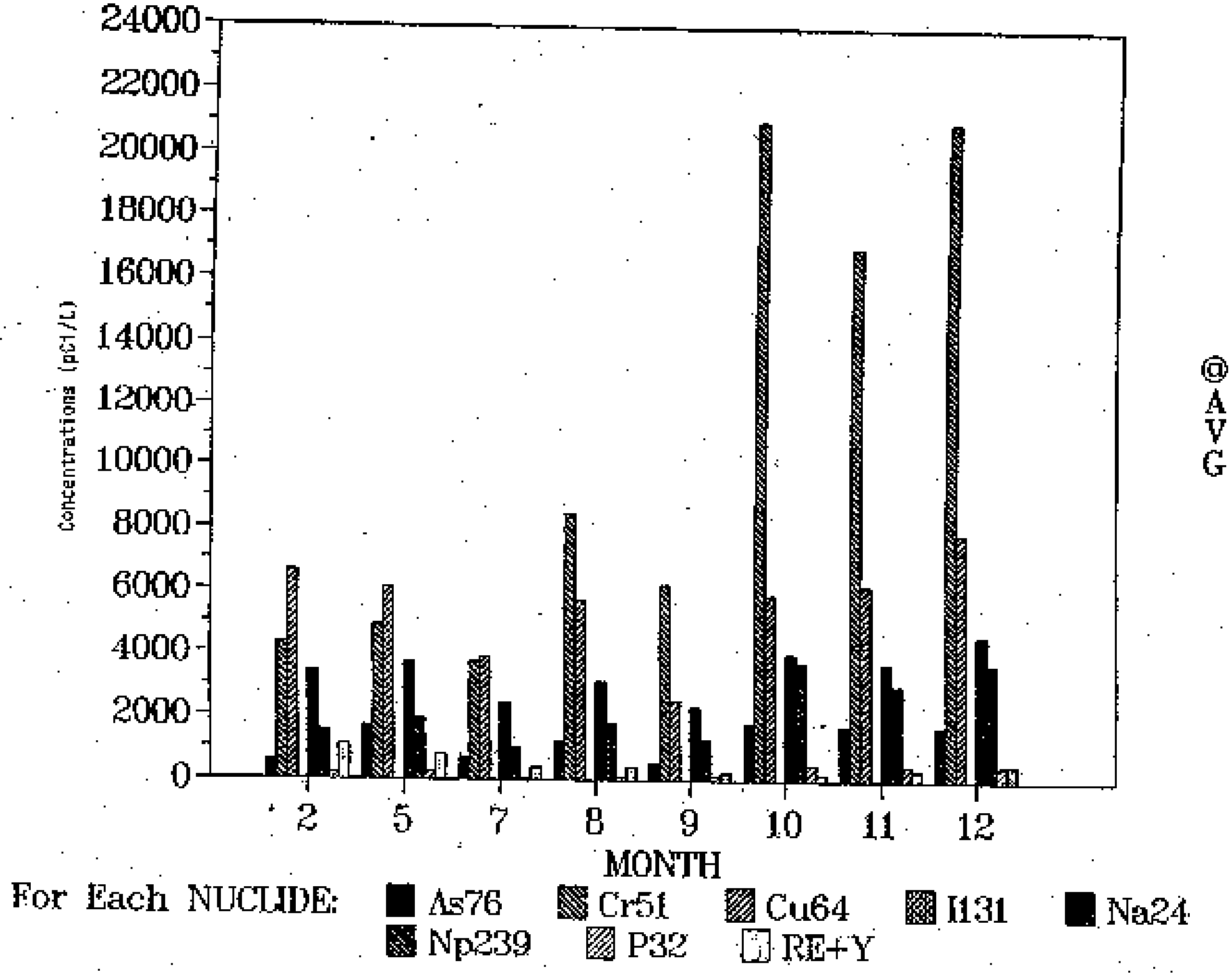

Figure 3-3. Monthly concentrations for selected radionuclides in Columbia River grab samples, 1963 (Dirkes, 1992; Nelson et al., 1964)

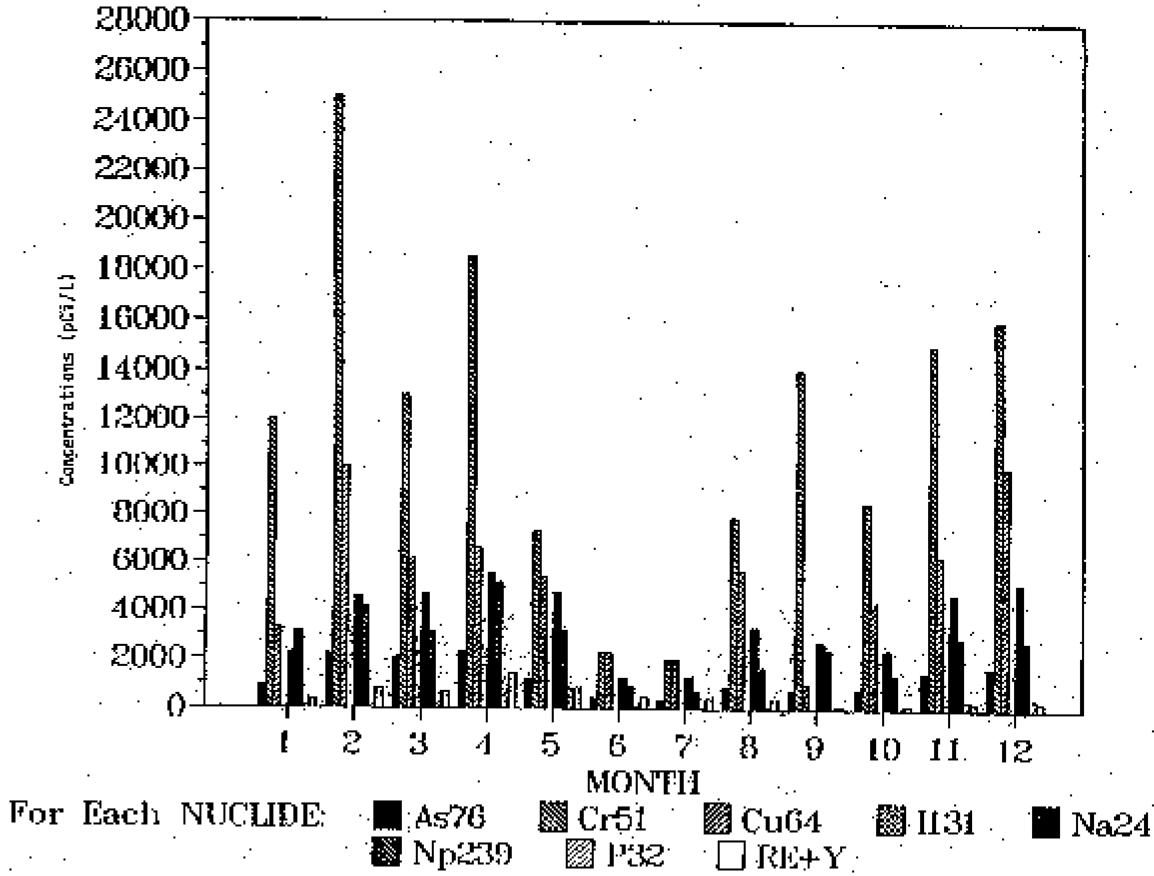

Figure 3-4. Monthly concentrations for selected radionuclides in Columbia River grab samples, 1964 (Dirkes, 1992; Nelson et al., 1964)

Table 3-3. Maximum Grab Sample Water Exposure Concentrations for 1963-1964 Time Period (Dirkes, 1992; Nelson et al., 1964)

Radionuclide	**Concentration (pCi/L)**
As-76	2,300
Co-60	120
Cr-51	25,000
Cu-64	10,000
I-131	34
Na-24	5,600
Np-239	5,600
P-32	630
RE+Y	1,400
Sr-90	2.6
Zn-65	1,800

3.3.3.4. Calculation of Organism Dose

The internal total-body dose rate to an organism from water exposure for a number (N) of radionuclides is given as:

$$R_c = \sum_{i=1}^{N} b_{i,c} E_{i,c} \tag{3-1}$$

where R_c is the dose rate to total body of organism c (rad d^{-1}), $b_{i,c}$ is the specific body burden of nuclide i in organism c (Bq kg^{-1}), and $E_{i,c}$ is the effective absorbed energy rate for nuclide i per unit activity in organism c (rad Ci^{-1} d^{-1}):

$$E_{i,c} = \epsilon_{i,c}\text{MeV dis}^{-1} \times 3.70\text{E}10 \text{ dis s}^{-1} \text{ Ci}^{-1}$$

$$86{,}400 \text{ sd}^{-1} \times 1.602\text{E-}11 \text{ rad}^{-1} \text{ MeV} = 5.12\text{E}4\ \epsilon_{i,c}$$

(where ϵ is the effective absorbed energy for nuclide i in organism c).

For a primary organism:

$$b_{i,c} = C_{i,c} B_{i,c} \tag{3-2}$$

where $C_{i,c}$ is the concentration of nuclide i in the water to which organism c is exposed (Bq m^{-3}) and $B_{i,c}$ is the bioaccumulation factor for nuclide i and organism c (m^3 kg^{-1}). Here the water concentration already has been corrected for dilution and radioactive decay during transit from the point of release into the receiving water body to the region of the organism's habitat.

Combining equations 3-1 and 3-2 yields the dose rate in rad/d to the primary organism, as shown in equation 3-3 below. The calculation of internal dose from tissue concentration is the same as equations 3-1 and 3-2, except the radionuclide-specific BCF is not used and correction for decay and dilution is unnecessary.

$$R_c = \sum_{i=1}^{N} C_{i,c} B_{i,c} \varepsilon_{i,c} \qquad (3\text{-}3)$$

For a secondary organism, such as an herbivore or carnivore, an expression can be written for a single radionuclide equating the change in body burden to the uptake and removal of the radionuclide.

3.3.3.5. Dose From Water Exposure

Table 3-4 shows the CRITR2 code calculations of organism dose from water exposure to various radionuclides. Appendix B provides a more detailed listing of CRITR2 code calculations and bioaccumulation factors used. Water concentrations were maximum values for the 2-year period. Table 3-4 indicates internal dose, immersion or surface dose (external water dose), and sediment dose. Internal exposure gave the maximum dose. Since immersion and sediment doses made only minor contributions, they were not considered in the risk characterization.

Table 3-4 summarizes dose for each organism. CRITR2 default organisms are generic plants, fish, crayfish, and ducks that eat plants and fish (DUCK-P and DUCK-F, respectively). Plant-eating ducks had the maximum dose rate, followed by plants, crayfish, fish, and fish-eating ducks. The dose rates to the plant-eating duck and crayfish exceeded the 1 rad/d level. The maximally exposed fish had a dose rate of 0.42 rad/d.

The dose to salmon eggs was estimated from measured river water radionuclide activities (table 3-3). Bioconcentration factors were estimated for salmon embryos from bioconcentration data reported for developing plaice (*Pleuronectes platessa*) embryos with respect to various fission product radionuclides (Woodhead, 1970). Concentration factors for day 4 of embryonic development ranged from <1 to 10 as a function of the radionuclide. This assessment used a whole egg concentration factor of 10 for all radionuclides shown in table 3-3. Dose calculations employed an overall egg diameter of 2 mm. Dose to whole eggs was 0.00442 rad/d.

3.3.3.6. Dose From Measured Tissue Concentrations

Table 3-5 lists calculated dose from measured tissue concentrations to selected organisms in the Columbia River. Phytoplankton had the highest dose at 14 rad/d, followed by limpet hard parts (shell) at 0.39 rad/d and caddisfly at 0.38 rad/d. The maximally exposed fish dose was calculated to be 0.73 rad/d. For fish, table 3-5 concentrations used to calculate dose represent the maximum values observed for whitefish during 1963-1964. Dose was evaluated for other species, but whitefish had the highest body dose for the study period. Unfortunately, most of the fish data were muscle tissue concentrations and therefore underestimated whole-body burdens. Consequently, the assessment adjusted these values to whole-body values. Based on limited Hanford data and published literature, the correction factors between whole body and muscle were 9:1 for phosphorus-32 and chromium-51 and 4:1 for zinc-65 (Poston and Strenge, 1989; U.S.

Table 3-4. CRITR2 Code Calculation of Organism Dose From Water Exposure to Various Radionuclides (Baker and Soldat, 1992)

```
OUT File Name:  RMAX.OUT    Created:  09:52   18-MAY-92
USR File Name and Header:  RMAX.USR      RMAX.USR    Columbia River Max Concentrations      18 May 92
Version of Program used:   V 1.0  of 26-Mar-92
```

****** CRITR2 -- Aquatic Biota Screening Dose Rates ******

TITLE: Columbia River Max Concentrations -- Ecological Risk Assessment

	Release	Plant	Fish	Crayfish	Duck-P	Duck-F
				Organism Dose Rates		
	Ci/y			Internal (rad/d)		
AS-76	---	3.9E-02	3.9E-02	3.9E-02	3.1E-03	6.1E-03
CO-60	---	2.7E-03	8.9E-04	2.9E-03	1.1E-03	7.3E-04
CR-51	---	2.7E-02	1.4E-04	7.1E-03	1.0E-02	1.0E-04
CU-64	---	1.6E-01	2.0E-01	2.8E-02	6.0E-03	1.5E-02
I-131	---	1.3E-04	2.1E-05	3.7E-05	1.4E-04	4.6E-05
NA-24	---	3.0E-02	3.0E-02	2.2E-02	2.6E-03	5.1E-03
NP-239	---	1.8E-02	1.5E-01	1.8E-03	6.2E-06	1.0E-04
P-32	---	1.1E+01	3.8E-03	2.2E+00	1.8E+01	1.2E-02
SR-90	---	4.6E-04	7.6E-06	1.5E-05	4.8E-03	1.6E-04
ZN-65	---	1.6E-01	5.0E-04	3.6E-02	1.6E+00	1.0E-02
Totals ------->		1.2E+01	4.2E-01	2.4E+00	1.9E+01	4.9E-02
	Ci/y			Immersion or Surface (rad/d)		
AS-76	---	3.0E-05	3.0E-05	1.5E-05	1.7E-05	1.7E-05
CO-60	---	9.3E-06	9.3E-06	4.6E-06	5.1E-06	5.1E-06
CR-51	---	2.4E-05	2.4E-05	1.2E-05	1.3E-05	1.3E-05
CU-64	---	5.6E-05	5.6E-05	2.8E-05	3.1E-05	3.1E-05
I-131	---	3.9E-07	3.9E-07	1.9E-07	2.1E-07	2.1E-07
NA-24	---	7.9E-04	7.9E-04	4.0E-04	4.4E-04	4.4E-04
NP-239	---	2.9E-05	2.9E-05	1.4E-05	1.6E-05	1.6E-05
P-32	---	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
SR-90	---	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
ZN-65	---	3.2E-05	3.2E-05	1.6E-05	1.8E-05	1.8E-05
Totals ------->		9.7E-04	9.7E-04	4.9E-04	5.3E-04	5.3E-04
	Ci/y			Sediment (rad/d)		
AS-76	---	2.9E-06	2.9E-06	5.9E-06	1.2E-06	1.2E-06
CO-60	---	1.8E-04	1.8E-04	3.5E-04	7.1E-05	7.1E-05
CR-51	---	6.5E-05	6.5E-05	1.3E-04	2.6E-05	2.6E-05
CU-64	---	2.8E-06	2.8E-06	5.7E-06	1.1E-06	1.1E-06
I-131	---	3.0E-07	3.0E-07	6.0E-07	1.2E-07	1.2E-07
NA-24	---	3.4E-05	3.4E-05	6.8E-05	1.4E-05	1.4E-05
NP-239	---	6.9E-06	6.9E-06	1.4E-05	2.8E-06	2.8E-06
P-32	---	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
SR-90	---	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
ZN-65	---	4.3E-04	4.3E-04	8.5E-04	1.7E-04	1.7E-04
Totals ------->		7.1E-04	7.1E-04	1.4E-03	2.9E-04	2.9E-04
Grand Totals >>>>>		1.2E+01	4.3E-01	2.4E+00	1.9E+01	5.0E-02

Table 3-4. CRITR2 Code Calculation of Organism Dose From Water Exposure to Various Radionuclides (continued)

OUT File Name: RMAX.OUT Created: 10:21 18-MAY-92

USR File Name: RMAX.USR

Version of Program used: V 1.0 of 26-Mar-92

Parameters and Water Concentrations

No dilution model used.

Bioaccumulation Factors for: Fresh No bioaccumulation factor corrections used.

	Release	Outfall Concentration	Plant	Fish	Crayfish	Duck-P	Duck-F
Distance (m) ------			1	1	1	1	1
Mixing Ratio ------			1	1	1	1	1
Radius (cm) -------			5.0	5.0	2.0	5.0	5.0
Mass (kg) ---------			--	--	--	1.0	1.0
Intake rate (g/d) -			--	--	--	100	200
Diet --------------			--	--	--	P	-F
Transit Time (h) --			0	0	0	0	0

Water Concentrations , (Decay during transit included)

	H. L.	--Ci/y-	Ci/m3 or uCi/mL					
AS-75	26.32 H	---	2.3E-06	2.3E-06	2.3E-06	2.3E-06	2.3E-06	2.3E-06
CO-60	5.271 Y	---	1.2E-07	1.2E-07	1.2E-07	1.2E-07	1.2E-07	1.2E-07
CR-51	27.704 D	---	2.5E-05	2.5E-05	2.5E-05	2.5E-05	2.5E-05	2.5E-05
CU-64	12.701 H	---	1.0E-05	1.0E-05	1.0E-05	1.0E-05	1.0E-05	1.0E-05
I-131	8.04 D	---	3.4E-08	3.4E-08	3.4E-08	3.4E-08	3.4E-08	3.4E-08
NA-24	15.00 H	---	5.6E-06	5.6E-06	5.6E-06	5.6E-06	5.6E-06	5.6E-06
NP-239	2.355 D	---	5.6E-06	5.6E-06	5.6E-06	5.6E-06	5.6E-06	5.6E-06
P-32	14.29 D	---	6.3E-07	6.3E-07	6.3E-07	6.3E-07	6.3E-07	6.3E-07
SR-90	29.12 Y	---	2.6E-09	2.6E-09	2.6E-09	2.6E-09	2.6E-09	2.6E-09
ZN-65	243.9 D	---	1.8E-06	1.8E-06	1.8E-06	1.8E-06	1.8E-06	1.8E-06

Table 3-5. Calculated Dose Based on Tissue Concentration for Selected Organisms of the Columbia River[a]

	Tissue Concentration (pCi/g wet weight)							
Organism	**Na-24**	**Cr-51**	**Mn-56**	**Cu-64**	**Zn-65**	**La-140**	**P-32**	**Total**
Plankton	1,414	59,500	291,000	102,000	14,000	5.900	23,000	14.8
Dose, rad/d	5.2E-2	6.8E-3	13.1	0.70	0.021	0.21	0.80	
Caddisfly	764	1,390	6,490	8,560	2,980			
Dose, rad/d	0.028	1.6E-4	0.29	0.058	3.1E-3			0.38
Chironimids	1,595	1,940	1,700	2,230	658	113		
Dose, rad/d	0.058	2.2E-4	0.076	0.015	9.7E-4	4.4E-3		0.15
Limpets (soft parts)	1,595	1,940	2,230	4,500	2,820	73		
Dose, rad/d	0.050	2.2E-4	0.076	0.031	4.2E-3	2.6E-3		0.17
Limpets (shell)	644	1,080	7,480	2,230	658	113		
Dose, rad/d	0.024	1.2E-4	0.35	0.015	9.7E-4	4.0E-3		0.39
Clams (soft parts)	393	620	556	3,320	1,100	47		
rad/d	0.014	7.0E-5	0.025	0.022	1.6E-3	1.7E-3		0.065
Clam (shell)	136	181	617	383	441	5		
Dose, rad/d	5.0E-3	2.1E-5	0.028	2.6E-3	6.5E-4	1.8E-4		0.036
Crayfish	955	536	982	48	811	12		
Dose, rad/d	0.035	6.1E-5	0.044	5.1E-3	1.2E-3	4.5E-4		0.085
Fish (whitefish)					270		20,700	
Dose, rad/d					1.7E-3		0.73	0.73

[a]Using minimum effective radius of 1.4 cm.

Table 3-6. Maximum Sediment Radionuclide Concentrations in the Hanford Reach and Dose to an Organism Living in the Sediments (Dirkes, 1992; Haushild et al., 1966; Nelson et al., 1964)

Nuclide	Concentration (pCi/Kg dry weight)[a]
Cr-51	13,000
Co-60	100
Sc-46	46
Zn-65	3,900

[a]Total dose: Organism buried in sediment—0.16 rad/d.
Organism on surface of sediment—0.08 rad/d.

Congress, Joint Committee on Atomic Energy, 1959). This correction introduces uncertainty into the effects characterization, but uncorrected muscle values could underestimate individual dose.

3.3.3.7. Dose From Measured Sediment Concentrations

Table 3-6 shows the calculated dose from exposure to radioactivity reported in sediments of the Columbia River. The calculated dose was quite small compared with other pathways.

Comments on Characterization of Exposure

Strengths of the case study include:

- *The ability to evaluate the worst case (maximally exposed individual) at the most sensitive life stage is an efficient method of screening for population-level effects. This study also benefits from the availability of long-term data sets collected on site.*

Limitations include:

- *Analysis also should consider potential uptake from food rather than only exposure or direct uptake from the water. Large variance in BCF values suggest that activity in water cannot reliably predict exposure.*
- *In the computer model scenario, algae, crayfish, and fish were not growing or eating and did not accumulate a food chain dose. Although the computer code included ducks, they were not included in the ecological risk assessment because of limited data and limited ability to verify the model estimate.*

3.3.4. Analysis: Characterization of Ecological Effects

Characterization of effects was based on dose-response information for fish from available toxicity data and also on regulatory standards. Conducted at the individual level, the characterization was interpreted qualitatively and applied to the population level of ecological organization.

The general response of aquatic organisms to ionizing radiation occurs at both the cellular and biochemical levels. Environmental factors also can affect the level of response. An NCRP (1991) report, *Effects of Ionizing Radiation on Aquatic Organisms*, provided the basis for stressor-response relationships developed in this report. Figures 3-5 and 3-6 were adapted from the NCRP report and summarize the information on acute effects of ionizing radiation on aquatic organisms.

One would expect different fish species to accumulate different concentrations of radionuclides based on their feedings habits, age, length of time spent at the site, and other factors. Depending on the level of exposure, mortality can occur. The threshold level of radiation dose that can cause acute mortality occurs at approximately 100 rad (1 Gy) for amphibians and 1,000 rad (10 Gy) for crustaceans and fish (figure 3-5). Figure 3-5 summarizes the relationship between organism dose and response and also shows the range for LD_{50}s. Under no circumstances did calculated dose to fish or other organisms exceed the boundary dose where acute effects would be observed. Dose calculations based on tissue concentrations for selected Columbia River organisms confirmed this finding. No aquatic animal organism used in the risk assessment exceeded the DOE dose limit of 1 rad/d.

Few studies have evaluated the effects of chronic exposure to ionizing radiation. However, it is known that the early developmental stages of chinook salmon are especially sensitive to ionizing radiation. NCRP (1991) reported that exposure to 5.1 rad/d (51 mGy/d) for up to 69 days produced no increase in mortality to chinook salmon embryos and alevins up to release as smolts. Hershberger et al. (1978) reported lower return of spawning adult chinook salmon after exposure of eggs and alevins at approximately 10 rad/d of gamma radiation. Gonadal development was retarded in chinook salmon on exposure to 10 rad/d delivered to embryos (Bonham and Donaldson, 1972). Other laboratory research (Erickson, 1973) found that an exposure of 0.4 rad/d (4.0 mGy/d) reduced courting activity for male *Poecilia reticulata* exposed as embryos. Chronic gamma radiation (190 days at an exposure of 18.5 rad/d) causes sterility in young adult *Ameca splendens* (Rackham and Woodhead, 1984).

Based on available literature, the dose used in DOE Order 5400.5 appears sufficiently conservative to protect most aquatic organisms. Consequently, unless future data indicate otherwise, this dose can be considered protective of populations and the ecosystem in general. To date, the sole qualifier is the work of Erickson (1973), who reported reduced male guppy courting activity when exposed to 0.4 rad/d. Little other information exists with regard to behavioral changes in fish exposed to ionizing radiation.

Figure 3-6 summarizes the effects of acute irradiation on development of fish. The threshold for developmental effects on fish occurs at approximately 5 rad (0.05 Gy), as observed for the one-cell-stage developing chinook salmon embryos. Radiosensitivity reportedly decreases

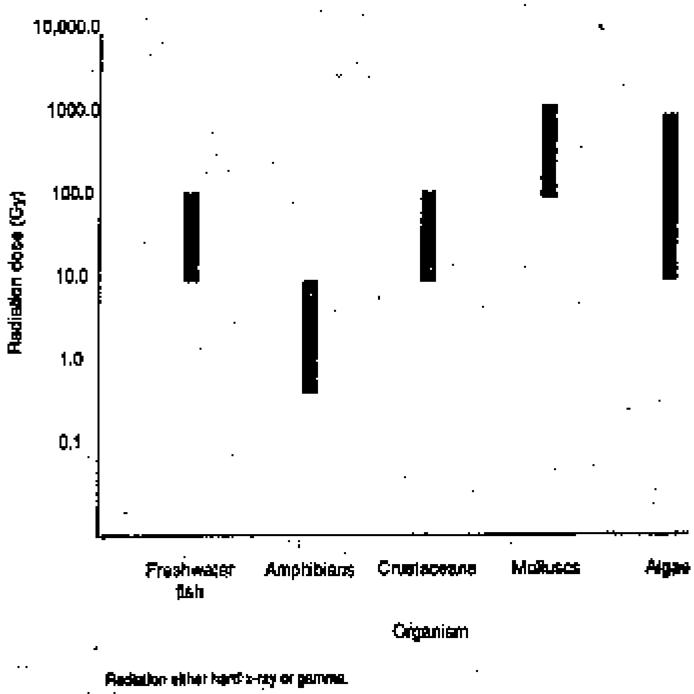

Figure 3-5. Ranges of sensitivities of aquatic organisms to acute radiation exposure (adapted from NCRP, 1991)

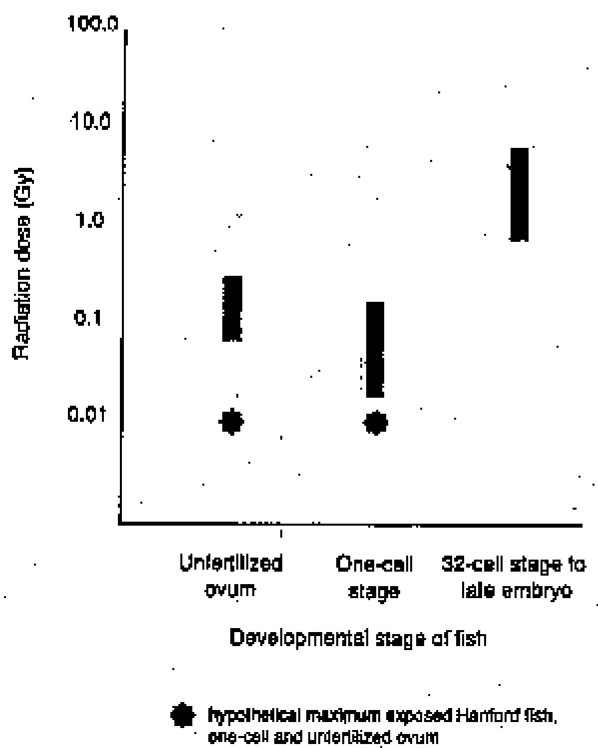

Figure 3-6. Ranges of sensitivities of the early developmental stages of fish to acute exposures (adapted from NCRP, 1991)

with increasing level of embryo development (Frank, 1973). Laboratory studies with the chinook salmon identify early life stages as the most sensitive for fish. Damage occurred when the dose reached 9.64 rad/d (4 mGy/h) over an 81-day development period (Hyodo-Taguchi, 1980). Studies have shown that 224 rad (2.24 Gy) reduced female germ cells in chinook salmon; a dose of 600 rad (6 Gy) produced the same effect in rainbow trout.

Comments on Characterization of Ecological Effects

Strengths of the case study include:

- *Direct experimental observations (dose-response curves) were provided to characterize effects. Figures 3-5 and 3-6 include ranges of acute toxicity data for various taxonomic groups and different life stages of salmon.*

General reviewer comments:

- *It was suggested that more sensitive measures than mortality should be used to assess effects. Dose-response curves could be provided to indicate the conservative nature of the DOE regulatory limit.*

- *No data are presented to show that protecting salmon embryos protects the ecosystem.*

3.3.5. Risk Characterization

Ecological risk was characterized by assessing dose to fish and, as indicators of ecosystem integrity, other aquatic organisms; by comparing doses to DOE Order 5400.5; and by comparing doses to published toxicity data.

3.3.5.1. Acute Exposure to Ionizing Radiation

The level of potential risk from ionizing radiation was assessed for fish under both acute and chronic exposure scenarios. The acute exposure considered mortality, while chronic exposure considered developmental effects as measurement endpoints.

To determine the potential risk to fish, both water and organism concentrations of radionuclides were converted to dose (tables 3-4 and 3-5, respectively). A comparison of these values (0.43 and 0.73 rad/d) to the range of acute toxicity (LD_{50}) reported for fish shows that no acute mortality would be expected from these levels. To assess exposure effects on a developing embryo, the whole egg dose was calculated to be 0.00442 rad/d.

The characterization of the level of potential risk to fish during early developmental stages and as adults was expressed as a hazard quotient (HQ), defined as the ratio of radionuclide organism dose (exposure or tissue value) to a dose-response benchmark value:

$$HQ = \frac{\text{Exposure Dose}}{\text{Dose Benchmark Value}} \qquad (3\text{-}4)$$

If the HQ is equal to or greater than 1, the likelihood of an adverse effect or high risk exists. The characterization was completed for the maximally exposed individual for the study period. It was assumed that if risk to the individual was low, the population was not at risk.

The hazard quotients shown in table 3-7 for early developmental stages of fish and adults were compared with toxicity values and DOE Order 5400.5. The maximum hazard quotient was 0.73 for adult fish. Assuming that this was the maximally exposed individual, the likelihood of an adverse effect to an individual was low.

Table 3-7. Hazard Quotient for Early Development Stage of Fish and Adult Fish

	Maximum Exposure	Minimum Effect Level	Hazard Quotient
Unfertilized ovum, One-cell stage	0.00442	0.96,[a] 0.4[b]	0.004,[a] 0.11[b]
Adult	0.73	1	0.73[a,c]

[a]Based on recommendation of the NCRP (1991).
[b]Based on male courting activity in guppies (Erickson, 1973).
[c]DOE Order 5400.5.

3.3.5.2. Chronic Exposure to Ionizing Radiation

Mortality from chronic exposure presented minimal risk to fish. Chronic exposure to 5.1 rad/d for up to 69 days did not produce any mortality to chinook salmon embryos or alevins (NCRP, 1991). Hershberger et al. (1978) reported lower return of spawning chinook salmon after exposure of eggs and alevins to 10 rad/d and effects on gonadal development in chinook salmon was reported to occur at 9.5 rad/d. Because the maximum dose rate to Columbia River adult fish and developing embryos was 0.73 and 0.00442 rad/d respectively, no chronic effects or mortality would be expected. Applying the behavior response noted for guppy embryo exposure (Erickson, 1973), the benchmark concentration would be 0.4 rad/d with an HQ of 0.1.

3.3.5.3. Uncertainty

Extrapolation of individual effects of radionuclides to populations and communities suffers from the same constraints as similar extrapolations for hazardous chemicals. The quantitative relationship between potential effects to fish or fish embryos and population and community response is not known. However, the effects data available for radionuclides showed that the single-cell stage in salmon is one of the more sensitive indicators of irradiation effects in fish and that protection of this stage of development should be protective of the population. Although specific data were not available for salmon embryo, data for embryo development of plaice was used to estimate dose.

The NCRP (1991) suggests that a "maximum dose rate 0.4 mGy/h (0.96 rad/d) would provide protection for endemic populations of aquatic organisms in environments receiving discharges of radioactive effluent." It further states, "adoption of a reference level of 0.4 mGy/h appears to represent a reasonable compromise based on current literature, i.e., considering both the nature of the effects observed at this dose rate and the limited amount of information on effects of radiation in natural populations, including interactions between ionizing radiation and ecological conditions." This value is also in agreement with DOE Order 5400.5.

Because whitefish are resident species in the Columbia River and can accumulate radionuclides throughout their life cycle, the assessment assumed that the whitefish tissue dose would be sufficiently conservative to extrapolate dose levels to other adult fish, including salmon. Salmon, on the other hand, spend only a short period of time in the river and do not feed when present. In addition, during the spring and early fall when salmon are present, river concentrations of radionuclides were generally the lowest.

The risk characterization used the maximally exposed individual to calculate organism dose. The risk characterization assumed that if an organism dose is below any known effect level with some degree of certainty, then the likelihood of an adverse effect is minimal. (The assessment endpoint was maintenance of important recreational fish populations in the Columbia River measured by protection of fish populations and specifically salmon embryos.) Results indicate that this is a reasonable assumption. Fish appear to be a suitable choice of receptor for screening risk from ionizing radiation. In addition, a fish dose of less than 1 rad/d should be protective of the ecosystem in general. However, since CRITR2 indicate that ducks could have received a dose higher than 1 rad/d, further studies are warranted.

Another area of uncertainty in the risk assessment is the extrapolation of muscle tissue concentration to whole fish concentrations for radionuclides. The assumption that protection of the maximally exposed individual extrapolated to sensitive life stages constitutes an adequate measure of the assessment endpoint also is a source of uncertainty. Alternatively, the hazard quotient is a reasonable approach for radionuclides for baseline or screening assessments.

3.3.5.4. Conclusions

This study demonstrates that the ecological risk assessment paradigm is applicable to radioactive substances. However, stressor-response data were limited to acute exposures; few data addressed chronic sublethal exposures. Most endpoints used for hazardous chemicals are expected to be equally appropriate for radionuclides. This study uncovered only one benchmark that specifically addressed protecting aquatic organisms from exposure to radiation. DOE Order 5400.5 limits exposure to aquatic animals to 1 rad/d.

Risk characterization did not indicate any measurable risk to the most sensitive aquatic organism (early life stage of chinook salmon) from exposure to radionuclides in sediments or water in the Columbia River. During peak production at Hanford, releases of radionuclides to the river did not result in a dose to fish that would exceed those specified in DOE Order 5400.5.

Dose calculations for radionuclide exposure from water and tissue concentrations provide for two methods for assessing the potential risks. This study investigated both methods and found that both provided reasonable results for fish, algae, and crayfish. Areas of uncertainty included the relationship between muscle and whole fish concentrations, the lack of a strong data base for organism exposure to chronic radiation, and a quantitative measure of ecosystem-level response to radionuclides. During the study period, the major thrust of monitoring at Hanford was to protect human health. Few studies examined ecosystem structure and function. Another significant area of uncertainty was the use of adult whitefish tissue concentration as a surrogate for chinook salmon. The study located no data suggesting that salmon accumulate a higher dose than whitefish, which spend their whole lives in the Columbia River. Although using fish data tends to increase uncertainty, fish are particularly sensitive to ionizing radiation and should provide a reasonable level of protection for fish populations and communities (figures 3-5 and 3-6) and a screen or benchmark indicator of ecosystem-level effects.

Comments on Risk Characterization

Strengths of the case study include:

- *The case study provides an opportunity to distinguish between screening assessments and more rigorous (realistic) assessments. The CRITR2 computer model is intended to provide a first pass that can be refined if there appear to be significant concerns.*

Limitations include:

- *The hazard quotient should be described in more detail by addressing the potential range of values, the establishment of confidence intervals, the degree of confidence that the value of 1.00 is safe, etc. This study uses the most sensitive individual to be conservative, but the selection of the most sensitive or highest exposed individual biases the assessment. The establishment of confidence bounds would result in a less biased measure of uncertainty.*
- *Many assumptions are chained together in this case study to obtain highly conservative assessments. A table should be developed that specifies these assumptions and the types of uncertainties they introduce.*
- *The focus on salmon limits an extrapolation to overall ecosystem effects.*

Comments on Risk Characterization (continued)

General reviewer comments:

- ***!*** *This section should emphasize that risk to the salmon populations is based on an analysis of risk to the most sensitive individuals and that risk from chemical exposure or other stressors was not evaluated. Nevertheless, risk from radionuclides is addressed adequately.*
- ***!*** *It would be helpful to have additional emphasis placed on estimating and using variability and confidence intervals. This could be the primary content for the section on uncertainty analysis.*

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APPENDIX A

COLUMBIA RIVER FISH SPECIES AND FOOD WEB

Table 3-A1. Fish Species in the Hanford Reach of the Columbia River

Common Name	Scientific Name
White sturgeon	*Acipenser transmontanus*
Bridgelip sucker	*Catostomus columbianus*
Largescale sucker	*Catostomus macrocheilus*
Mountain sucker	*Catostomus platyrhynchus*
Pumpkinseed	*Lepomis gibbosus*
Bluegill	*Lepomis macrochirus*
Smallmouth bass	*Micropterus dolomieui*
Largemouth bass	*Micropterus salmoides*
White crappie	*Pomoxis annularis*
Black crappie	*Pomoxis nigromaculatus*
American shad	*Alosa sapidissima*
Prickly sculpin	*Cottus asper*
Mottled sculpin	*Cottus bairdi*
Piute sculpin	*Cottus beldingi*
Reticulate sculpin	*Cottus perplexus*
Torrent sculpin	*Cottus rotheus*
Chiselmouth	*Acrocheilus alutaceus*
Carp	*Cyprinus carpio*
Peamouth	*Mylocheilus caurinus*
Northern squawfish	*Ptychocheilus oregonensis*
Longnose dace	*Rhinichthys cataractae*
Leopard dace	*Rhinichthys falcatus*
Speckled dace	*Rhinichthys osculus*
Redside shiner	*Richardsonius balteatus*
Tench	*Tinca tinca*
Burbot	*Lota lota*

Table 3-A1. Fish Species in the Hanford Reach of the Columbia River (continued)

Common Name	Scientific Name
Threespine stickleback	*Gasterosteus aculeatus*
Black bullhead	*Ictalurus melas*
Yellow bullhead	*Ictalurus natalis*
Brown bullhead	*Ictalurus nebulosus*
Channel catfish	*Ictalurus punctatus*
Yellow perch	*Perca flavescens*
Walleye	*Stizostedion vitreum vitreum*
Sand roller	*Percopsis transmontana*
Pacific lamprey	*Entosphenus tridentatus*
River lamprey	*Lampetra ayresi*
Lake whitefish	*Coregonus clupeaformis*
Coho salmon	*Oncorhynchus kisutch*
Sockeye salmon	*Oncorhynchus nerka*
Chinook salmon	*Oncorhynchus tshawytscha*
Mountain whitefish	*Prosopium williamsoni*
Cutthroat trout	*Oncorhynchus clarki*
Rainbow trout (steelhead)	*Oncorhynchus mykiss*
Dolly Varden trout	*Salvelinus malma*

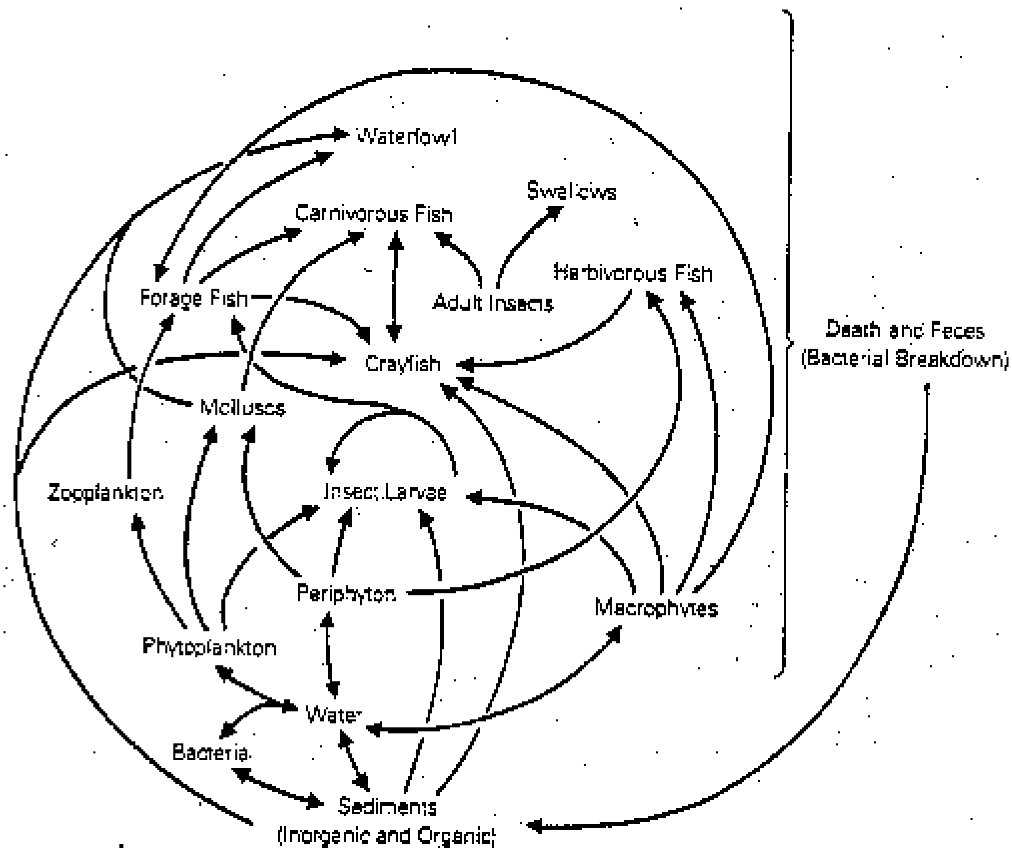

Figure 3-A1. Columbia River aquatic ecosystem

APPENDIX B

CRITR2 CODE CALCULATIONS AND BIOACCUMULATION FACTORS

CRITR Code Calculation of Organism Dose from Water Exposure to Various Radionuclides

CRITR QA Printout --- User File: RMAX.USR   Run of: 09:52   18-MAY-92

No Dilution Model used.

DFSWIM	DFSED	FSOLD	FRUF	TB	BUILDUP	TTRANS	EXP
3.5E-12	3.2E-11	6.9E-02	0.2	3.7E+02	1.6E+00	0.0E+00	1.0E+00

INUC	K	NUKSYMS	NS	CONCRIT	BIO	KB	RINTAKE	ECRIT	TBIO	F1	LAMC	MASS	DOSECRIT		
1	1	AS-76	P	8.5E+04	3.0E-01	1.000	0.000	1.5E-08	280.0	5.0E-01	0.0E+00	0.0	3.9E-04	PLANT	eats -
1	2	AS-76	F	8.5E+04	3.0E-01	1.000	0.000	1.5E-08	280.0	5.0E-01	0.0E+00	0.0	3.9E-04	FISH	eats -
1	3	AS-76	C	8.5E+04	3.0E-01	1.000	0.000	1.5E-08	280.0	5.0E-01	0.0E+00	0.0	3.9E-04	CRAWDAD	eats -
1	4	AS-76	P	8.5E+04	3.0E-01	1.000	0.100	1.5E-08	280.0	5.0E-01	6.3E-01	1.0	3.1E-05	DUCK-P	eats P
1	5	AS-76	F	8.5E+04	3.0E-01	1.000	0.200	1.5E-08	280.0	5.0E-01	6.3E-01	1.0	6.1E-05	DUCK-F	eats F

DFSWIM	DFSED	FSOLD	FRUF	TB	BUILDUP	TTRANS	EXP
2.1E-11	1.7E-10	6.9E-02	0.2	3.7E+02	3.4E+02	0.0E+00	1.0E+00

INUC	K	NUKSYMS	NS	CONCRIT	BIO	KB	RINTAKE	ECRIT	TBIO	F1	LAMC	MASS	DOSECRIT		
2	1	CO-60	P	4.4E+03	1.0E+00	1.000	0.000	6.0E-09	9.5	3.0E-01	0.0E+00	0.0	2.7E-05	PLANT	eats -
2	2	CO-60	F	4.4E+03	3.3E-01	1.000	0.000	6.0E-09	9.5	3.0E-01	0.0E+00	0.0	5.9E-06	FISH	eats -
2	3	CO-60	C	4.4E+03	2.0E+00	1.000	0.000	3.3E-09	9.5	3.0E-01	0.0E+00	0.0	2.9E-05	CRAWDAD	eats -
2	4	CO-60	P	4.4E+03	1.0E+00	1.000	0.100	6.0E-09	9.5	3.0E-01	7.3E-02	1.0	1.1E-05	DUCK-P	eats P
2	5	CO-60	F	4.4E+03	3.3E-01	1.000	0.200	6.0E-09	9.5	3.0E-01	7.3E-02	1.0	7.3E-06	DUCK-F	eats F

DFSWIM	DFSED	FSOLD	FRUF	TB	BUILDUP	TTRANS	EXP
2.6E-13	2.5E-12	6.9E-02	0.2	3.7E-02	4.0E+01	0.0E+00	1.0E+00

INUC	K	NUKSYMS	NS	CONCRIT	BIO	KB	RINTAKE	ECRIT	TBIO	F1	LAMC	MASS	DOSECRIT		
3	1	CR-51	P	9.3E+05	4.0E+00	1.000	0.000	7.3E-11	616.0	1.0E-01	0.0E+00	0.0	2.7E-04	PLANT	eats -
3	2	CR-51	F	9.3E+05	2.0E-02	1.000	0.000	7.3E-11	616.0	1.0E-01	0.0E+00	0.0	1.4E-06	FISH	eats -
3	3	CR-51	C	9.3E+05	2.0E+00	1.000	0.000	3.8E-11	616.0	1.0E-01	0.0E+00	0.0	7.1E-05	CRAWDAD	eats -
3	4	CR-51	P	9.3E+05	4.0E+00	1.000	0.100	7.3E-11	616.0	1.0E-01	2.6E-02	1.0	5.0E-04	DUCK-P	eats P
3	5	CR-51	F	9.3E+05	2.0E-02	1.000	0.200	7.3E-11	616.0	1.0E-01	2.6E-02	1.0	1.0E-06	DUCK-F	eats F

DFSWIM	DFSED	FSOLD	FRUF	TB	BUILDUP	TTRANS	EXP
1.5E-12	1.4E-11	6.9E-02	0.2	3.7E+02	7.6E-01	0.0E+00	1.0E+00

INUC	K	NUKSYMS	NS	CONCRIT	BIO	KB	RINTAKE	ECRIT	TBIO	F1	LAMC	MASS	DOSECRIT		
4	1	CU-64	P	3.7E+05	2.0E+00	1.000	0.000	2.1E-09	80.0	5.0E-01	0.0E+00	0.0	1.6E-03	PLANT	eats -
4	2	CU-64	F	3.7E+05	2.5E+00	1.000	0.000	2.1E-09	80.0	5.0E-01	0.0E+00	0.0	2.0E-03	FISH	eats -
4	3	CU-64	C	3.7E+05	4.0E-01	1.000	0.000	1.9E-09	80.0	5.0E-01	0.0E+00	0.0	2.8E-04	CRAWDAD	eats -
4	4	CU-64	P	3.7E+05	2.0E+00	1.000	0.100	2.1E-09	80.0	5.0E-01	1.3E+00	1.0	6.0E-05	DUCK-P	eats P
4	5	CU-64	F	3.7E+05	2.5E+00	1.000	0.200	2.1E-09	80.0	5.0E-01	1.3E+00	1.0	1.5E-04	DUCK-F	eats F

DFSWIM	DFSED	FSOLD	FRUF	TB	BUILDUP	TTRANS	EXP
3.1E-12	3.0E-11	6.9E-02	0.2	3.7E+02	1.2E+01	0.0E+00	1.0E+00

INUC	K	NUKSYMS	NS	CONCRIT	BIO	KB	RINTAKE	ECRIT	TBIO	F1	LAMC	MASS	DOSECRIT		
5	1	I-131	P	1.3E+03	3.0E-01	1.000	0.000	3.4E-09	100.0	1.0E+00	0.0E+00	0.0	1.3E-06	PLANT	eats -
5	2	I-131	F	1.3E+03	5.0E-02	1.000	0.000	3.4E-09	100.0	1.0E+00	0.0E+00	0.0	2.1E-07	FISH	eats -
5	3	I-131	C	1.3E+03	1.0E-01	1.000	0.000	2.9E-09	100.0	1.0E+00	0.0E+00	0.0	3.7E-07	CRAWDAD	eats -
5	4	I-131	P	1.3E+03	3.0E-01	1.000	0.100	3.4E-09	100.0	1.0E+00	9.3E-02	1.0	1.4E-06	DUCK-P	eats P
5	5	I-131	F	1.3E+03	5.0E-02	1.000	0.200	3.4E-09	100.0	1.0E+00	9.3E-02	1.0	4.6E-07	DUCK-F	eats F

DFSWIM	DFSED	FSOLD	FRUF	TB	BUILDUP	TTRANS	EXP
3.8E-11	2.6E-10	6.9E-02	0.2	3.7E+02	9.0E-01	0.0E+00	1.0E+00

INUC	K	NUKSYMS	NS	CONCRIT	BIO	KB	RINTAKE	ECRIT	TBIO	F1	LAMC	MASS	DOSECRIT		
6	1	NA-24	P	2.1E+05	1.0E-01	1.000	0.000	1.5E-08	11.0	1.0E+00	0.0E+00	0.0	3.0E-04	PLANT	eats -
6	2	NA-24	F	2.1E+05	1.0E-01	1.000	0.000	1.5E-08	11.0	1.0E+00	0.0E+00	0.0	3.0E-04	FISH	eats -
6	3	NA-24	C	2.1E+05	1.0E-01	1.000	0.000	1.1E-08	11.0	1.0E+00	0.0E+00	0.0	2.2E-04	CRAWDAD	eats -
6	4	NA-24	P	2.1E+05	1.0E-01	1.000	0.100	1.5E-08	11.0	1.0E+00	1.2E+00	1.0	2.6E-05	DUCK-P	eats P
6	5	NA-24	F	2.1E+05	1.0E-01	1.000	0.200	1.5E-08	11.0	1.0E+00	1.2E+00	1.0	5.1E-05	DUCK-F	eats F

DFSWIM	DFSED	FSOLD	FRUF	TB	BUILDUP	TTRANS	EXP
1.4E-12	1.4E-11	6.9E-02	0.2	3.7E+02	3.4E+00	0.0E+00	1.0E+00

INUC	K	NUKSYMS	NS	CONCRIT	BIO	KB	RINTAKE	ECRIT	TBIO	F1	LAMC	MASS	DOSECRIT		
7	1	NP-239	P	2.1E+05	3.0E-01	1.000	0.000	2.9E-09	39000.0	1.0E-03	0.0E+00	0.0	1.8E-04	PLANT	eats -
7	2	NP-239	F	2.1E+05	2.5E+00	1.000	0.000	2.9E-09	39000.0	1.0E-03	0.0E+00	0.0	1.5E-03	FISH	eats -
7	3	NP-239	C	2.1E+05	3.0E-02	1.000	0.000	2.8E-09	39000.0	1.0E-03	0.0E+00	0.0	1.0E-05	CRAWDAD	eats -
7	4	NP-239	P	2.1E+05	3.0E-01	1.000	0.100	2.9E-09	39000.0	1.0E-03	2.9E-01	1.0	6.2E-08	DUCK-P	eats P
7	5	NP-239	F	2.1E+05	2.5E+00	1.000	0.200	2.9E-09	39000.0	1.0E-03	2.9E-01	1.0	1.0E-06	DUCK-F	eats F

DFSWIM	DFSED	FSOLD	FRUF	TB	BUILDUP	TTRANS	EXP
0.0E+00	0.0E+00	6.9E-02	0.2	3.7E+02	2.1E+01	0.0E+00	1.0E+00

INUC	K	NUKSYMS	NS	CONCRIT	BIO	KB	RINTAKE	ECRIT	TBIO	F1	LAMC	MASS	DOSECRIT			
8	1	P-32	P	2.3E+04	5.0E-02	1.000	0.000	9.6E-09	257.0	8.0E-01	0.0E+00	0.0	1.1E-01	PLANT	eats	-
8	2	P-32	F	2.3E+04	1.7E-01	1.000	0.000	9.6E-09	257.0	8.0E-01	0.0E+00	0.0	3.8E-05	FISH	eats	-
8	3	P-32	C	2.3E+04	1.0E+02	1.000	0.000	9.6E-09	257.0	8.0E-01	0.0E+00	0.0	2.2E-02	CRAWDAD	eats	-
8	4	P-32	P	2.3E+04	5.0E+02	1.000	0.100	9.6E-09	257.0	8.0E-01	5.1E-02	1.0	1.8E-01	DUCK-P	eats	P
8	5	P-32	F	2.3E+04	1.7E-01	1.000	0.200	9.6E-09	257.0	8.0E-01	5.1E-02	1.0	1.2E-04	DUCK-F	eats	F

DFSWIM	DFSED	FSOLD	FRUF	TB	BUILDUP	TTRANS	EXP
0.0E+00	0.0E+00	6.9E-02	0.2	3.7E+02	3.6E-02	0.0E+00	1.0E+00

INUC	K	NUKSYMS	NS	CONCRIT	BIO	KB	RINTAKE	ECRIT	TBIO	F1	LAMC	MASS	DOSECRIT			
9	1	SR-90	P	9.6E+01	3.0E+00	1.000	0.000	1.6E-08	4000.0	3.0E-01	0.0E+00	0.0	4.6E-05	PLANT	eats	-
9	2	SR-90	F	9.6E+01	5.0E-02	1.000	0.000	1.6E-08	4000.0	3.0E-01	0.0E+00	0.0	7.6E-08	FISH	eats	-
9	3	SR-90	C	9.6E+01	1.0E-01	1.000	0.000	1.6E-08	4000.0	3.0E-01	0.0E+00	0.0	1.5E-07	CRAWDAD	eats	-
9	4	SR-90	P	9.6E+01	3.0E+00	1.000	0.100	1.6E-08	4000.0	3.0E-01	2.4E-04	1.0	4.8E-05	DUCK-P	eats	P
9	5	SR-90	F	9.6E+01	5.0E-02	1.000	0.200	1.6E-08	4000.0	3.0E-01	2.4E-04	1.0	1.6E-06	DUCK-F	eats	F

DFSWIM	DFSED	FSOLD	FRUF	TB	BUILDUP	TTRANS	EXP
4.8E-12	4.1E-11	6.9E-02	0.2	3.7E+02	2.3E+02	0.0E+00	1.0E+00

INUC	K	NUKSYMS	NS	CONCRIT	BIO	KB	RINTAKE	ECRIT	TBIO	F1	LAMC	MASS	DOSECRIT			
10	1	ZN-65	P	6.7E+04	2.0E+01	1.000	0.000	1.2E-09	933.0	5.0E-01	0.0E+00	0.0	1.6E-03	PLANT	eats	-
10	2	ZN-65	F	6.7E+04	6.4E-02	1.000	0.000	1.2E-09	933.0	5.0E-01	0.0E+00	0.0	5.0E-06	FISH	eats	-
10	3	ZN-65	C	6.7E+04	1.0E+01	1.000	0.000	5.3E-10	933.0	5.0E-01	0.0E+00	0.0	3.6E-04	CRAWDAD	eats	-
10	4	ZN-65	P	6.7E+04	2.0E+01	1.000	0.100	1.2E-09	933.0	5.0E-01	3.6E-03	1.0	1.6E-02	DUCK-P	eats	P
10	5	ZN-65	F	6.7E+04	6.4E-02	1.000	0.200	1.2E-09	933.0	5.0E-01	3.6E-03	1.0	[illegible]E-04	DUCK-F	eats	F

Notes & Units:

Hanford biofactors used.

No bioaccumulation factor corrections used.

LAMDA	Rad. Decay constant	1/d	CONCRIT	Conc. in Water	Bq/m3
DFSWIM	Immersion DF	Sv/d per Bq	BIO	Bioaccum factor	m3/kg
DFSED	Sediment DF	Sv/d per Bq	RINTAKE	Intake rate	kg/d
FSOLD	Nuclide sed. buildup rate	m3/m2-d	ECRIT	Energy absorbed	J/Bq-d
FRUF	Roughness factor	--	TBIO	Biological half time	d
TB	Sed. Buildup time	d	F1	Fraction to total body	--
BUILDUP	Sed. Buildup	Bq-d/Bq	LAMC	Effective decay const.	1/d
TTRANS	Transport time	d	MASS	Organism mass	kg
EXP	Fractional decay during trans.	--	DOSECRIT	Organism Dose	Gy/d

Hanford-Specific Bioaccumulation Factors and Human Biological Half-Lives and Uptake Fractions (Baker and Soldat, 1992)

	Fish		Crustacean		Mollusc		Plant		T_b	f_1
	Fresh	Salt	Fresh	Salt	Fresh	Salt	Fresh	Salt		
	L/kg								--d--	-----
As	300	300	300	300	300	300	300	300	280	0.5
Cr	20	600	2000	500	2000	1140	4000	4000	616	0.1
Cs	2000	100	100	30	100	30	500	700	115	1
Cu	2500	1000	400	5000	400	5000	2000	1000	80	0.5
Fe	2000	3000	100	5000	100	30000	1000	50000	800	0.1
Mn	400	400	100000	800	100000	6000	10000	10000	17	0.1
Na	100	1	100	0.1	100	0.3	100	1	11	1
Ni	100	100	500	500	500	500	500	3000	667	0.05
Np	2500	2500	30	10	30	150	300	6	39000	0.001
P	170	28000	100000	38000	1100000	45000	500000	00000	257	0.8
Sc	100	750	1000	300	1000	100000	10000	1000	30	1E-4
Zn	64	1000	10000	50000	10000	30000	20000	50000	933	0.5

SECTION FOUR

ECOLOGICAL RISK ASSESSMENT CASE STUDY:

EFFECTS OF PHYSICAL DISTURBANCE ON WATER QUALITY STATUS AND WATER QUALITY IMPROVEMENT FUNCTION OF URBAN WETLANDS

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LIST OF ACRONYMS

DP	dissolved phosphorus
DOC	dissolved organic carbon
EPA	Environmental Protection Agency
FTU	formazin turbidity units
FWS	Fish and Wildlife Service
MANOVA	multivariate analysis of variance
MN DNR	Minnesota Department of Natural Resources
MPCA	Minnesota Pollution Control Agency
NTU	nephelometric turbidity units
NWI	National Wetland Inventory
NWP	Nationwide Permit
PCU	platinum cobalt unit
SRP	soluble reactive phosphorus
TCMA	Twin Cities metropolitan area
TDS	total dissolved solids
TP	total phosphorus
TSS	total suspended solids
U.S. ACOE	U.S. Army Corps of Engineers

ABSTRACT

This case study demonstrates an empirical approach to quantifying the regional risk to the water quality of wetlands and adjacent surface waters based on the frequency, type, and intensity of physical disturbances. The case study describes an investigation, which began in the fall of 1988, to determine the effects of physical and hydrological modifications on wetland water quality function in the eight-county Minneapolis/St. Paul metropolitan area. Investigators identified the incidence of potential stressors to wetland water quality function through surveys of the U.S. Army Corps of Engineers (U.S. ACOE) 404 permits under the Clean Water Act, state and county agencies, and local watershed management organizations.

The study addressed 33 wetland sites potentially affected by deposition of fill, dredging, impoundment, sedimentation, and storm-water or pumped ground-water inputs during the succeeding year. Stressor intensities were quantified as wetland fill area, percentage wetland filled, change in water depth due to dredging or impoundment, changes in the ratio of watershed to wetland area, changes in the ratio of impervious surface area (urban or residential land use) to wetland area, and the ratio of construction area (bare earth) in the watershed to wetland area. Assessment endpoints were potential water quality effects relative to wetland biota (reduced transparency, altered ionic strength, low dissolved oxygen/high ammonia stress, and lead toxicity) and potential water quality impacts on downstream surface waters (eutrophication, reduced transparency, nitrate/nitrite toxicity, and lead toxicity). Measurement endpoints were changes between pre- and postdisturbance conditions in the following mid-wetland water quality parameters: temperature, dissolved oxygen, conductivity, turbidity, orthophosphate, nitrate plus nitrite, ammonia, dissolved and total nitrogen, phosphorus, organic carbon, total and volatile suspended solids, and total extractable lead. Sampling was conducted for up to 1 year prior to disturbance, during the peak-disturbance period, and over a 1- to 2-year postdisturbance or recovery period.

Investigators used a multiple regression approach to quantify stressor-response relationships. Change in a water quality variable between pre- and postdisturbance or recovery periods was regressed against measurements of disturbance intensity. The y-intercept in these regression equations represented annual changes in water quality in the absence of disturbance (e.g., due to interannual climate variability), while the slope of the relationship represented the response to increasing intensities of disturbance.

Risk characterization required integrating cause-effect relationships identified through site-specific investigations with information on regional distributions of stressor type and intensity. One of the greatest uncertainties associated with evaluating risks to wetland water quality in the study area was estimating the true incidence or intensity of unregulated or incompletely regulated physical or hydrologic disturbances, especially with respect to small, isolated headwater wetlands. Estimates of ecological risk to aquatic biota in wetlands also were hampered by problems in extrapolating water quality standards derived primarily for different classes of surface waters to wetlands.

4.1. RISK ASSESSMENT APPROACH

This case study represents a regional risk assessment of the impacts of physical and hydrological disturbance on the water quality status and function of freshwater emergent wetlands in the eight-county Minneapolis/St. Paul metropolitan area. The study was not designed to fit the complete U.S. Environmental Protection Agency (EPA) ecological risk assessment framework (U.S. EPA, 1992). In particular, investigators could not fully identify or quantify stressor characteristics during the problem formulation phase because of a lack of good background information. Thus, problem formulation was refined in conjunction with the stressor characterization portion of the analysis phase.

The study analyzed wetland water quality status and function, i.e., ecosystem-level effects. Ecological impacts on specific wetland biota were not the focus of the initial research, but investigators were able to analyze ecological risks to wetland biota and biota of downstream surface waters by comparing study area data with state water quality criteria and critical effects levels derived from the literature for relevant wetland biota (U.S. EPA, 1986). Figure 4-1 provides a summary of the assessment approach used.

4.2. STATUTORY AND REGULATORY BACKGROUND

One of the goals of the Clean Water Act is to restore and maintain the chemical, physical, and biological integrity of the waters of the United States. A panel of wetland experts broadly defined wetland integrity as ". . . the persistence of physical, chemical, and biological conditions that sustain the long-term processes and structure of the regional wetland resource . . ." (Adamus, 1989). Similarly, the Emergency Wetlands Resources Act of 1986 promotes "the conservation of the wetlands of the nation in order to maintain the public benefits they provide." Wetland-related activities within EPA focus on assessing and protecting wetland processes associated with water quality, flood control, and habitat functions of wetlands (Leibowitz et al., 1992).

In practice, the only federal regulatory framework consistently applied to protect wetlands is the program established under Section 404 of the Clean Water Act, which controls the disposal of dredge or fill material in wetlands. Much of the wetland fill activity in urbanizing areas was covered under Nationwide Permit (NWP) 26, which authorizes wetland fill of up to 10 acres in isolated or headwater wetlands, with no predischarge notification required for fill of less than 1 acre. Subsequently, as part of the 401 certification process, all NWP 26 applications filed in the State of Minnesota must include a predischarge notification (U.S. ACOE, 1992).

In spite of the wide range of disturbances to which wetlands are subjected (Leslie and Clark 1990), the assessment of long-term impacts on inland wetlands has been restricted to the loss of wetland area through fill or drainage (Tiner, 1984). Urban wetlands in particular are exposed to a wide range of physical modifications and hydrologic disturbances—filling, draining, dredging, impoundment, and storm-water or pumped ground-water inputs—yet little research or synthesis of information has been done to assess risks to these systems.

PROBLEM FORMULATION

Stressors: Partial fill, draining, dredging, impoundment, storm water, pumped ground water, and siltation.

Ecosystem(s) at Risk: Wetlands in the Twin Cities (MN) metropolitan area

Ecological Components: Submerged macrophyte communities, wetland habitats, threatened and endangered species.

Endpoints: Assessment endpoint is maintenance of wetland water quality function. Measurement endpoints are mid-wetland physical-chemical characteristics (e.g., suspended solids, specific conductivity, organic carbon).

ANALYSIS

Characterization of Exposure

Natural resource managers were surveyed and 404 permit notices reviewed. Indices of disturbance measured were: fill area, % wetland filled, changes in water depth, ratio of watershed, urban/residential, or construction area to wetland area.

Characterization of Ecological Effects

Mid-wetland water quality was measured each season. Changes among predisturbance, peak-disturbance, 1st year post-disturbance, and 2nd year recovery phase were calculated.

RISK CHARACTERIZATION

Peak- or postdisturbance water quality conditions were compared to the predisturbance phase. Mid-wetland water quality was compared to state surface water quality criteria and tolerance levels cited in literature for dominant wetland vegetation. Rate of recovery of mid-wetland water quality was assessed.

Figure 4-1. Structure of assessment for physical or hydrological impacts on wetland water quality status and function in the eight-county Minneapolis/St. Paul metropolitan area

4.3. CASE STUDY DESCRIPTION

4.3.1. Background Information and Objective

The antidegradation clause in the Clean Water Act requires the maintenance of wetland ecological integrity, while the Emergency Wetlands Resources Act promotes the conservation of public benefits (i.e., functions) of wetlands. A complete risk assessment of the impacts of physical or hydrologic disturbance on urban wetland status and function would require an examination of effects on wetland hydrologic functions (flood control, ground-water recharge), habitat functions, and water quality improvement functions.

Traditionally, the loss of wetland function has been monitored as a net change in wetland area (Dahl and Johnson, 1991). Minnesota's 1990 report to Congress under Section 305(b) of the Federal Water Pollution Control Act estimated that mitigation activities under the 404 permit program resulted in a statewide net loss of 61 acres of wetlands (of the 5.02 million acre total) during 1988-1989, with an additional 4,000 acres of wetlands restored or "enhanced" (MPCA, 1990). Similarly, a comparison of previous rates of wetland loss from drainage with recent rates shows a decrease. However, the loss of wetland function can occur through type conversions (with no loss of wetland area) as well as through degradation of existing conditions. Therefore, this risk assessment explicitly targets an information gap—the potential degradation of wetland water quality status and function due to common physical disturbances. Where possible, effects on wetland habitat (loss or conversion) are discussed, but quantification of these impacts was beyond the scope of this study.

The case study summarizes the results of a 3-year, $280,000 research project on the impacts on, and recovery of, mid-wetland water quality from physical or hydrologic disturbance in the eight-county Minneapolis/St. Paul metropolitan area. Stress-response curves derived from this study were supplemented with literature- and permit-based surveys of the incidence of physical or hydrologic disturbance activities in this region. Investigators also supplemented water quality criteria values with a literature review of tolerances of relevant wetland-dependent biota to measured water quality parameters. The Wetland Function Project (U.S. EPA Wetland Research Program) provided funding for the original research. At the time, the Wetland Function Project focused on wetland water quality and water quality functions; therefore, site investigations of potential habitat effects were limited to qualitative descriptions of dominant plant species or cover and to an assessment of changes in wetland type.

4.3.1.1. Study Area

The study area encompasses both the 7,330 km^2 Minneapolis/St. Paul metropolitan area and adjacent Wright County (figure 4-2). The population of the region is over 2,000,000, with the heaviest densities in the central cities of Minneapolis and St. Paul. Land use is 27 percent urban, 43 percent agricultural, and 30 percent open space (Ayers et al., 1985). Urbanization is rapidly spreading into agricultural and open areas, with greatest population increases now occurring in Anoka and northern Dakota Counties.

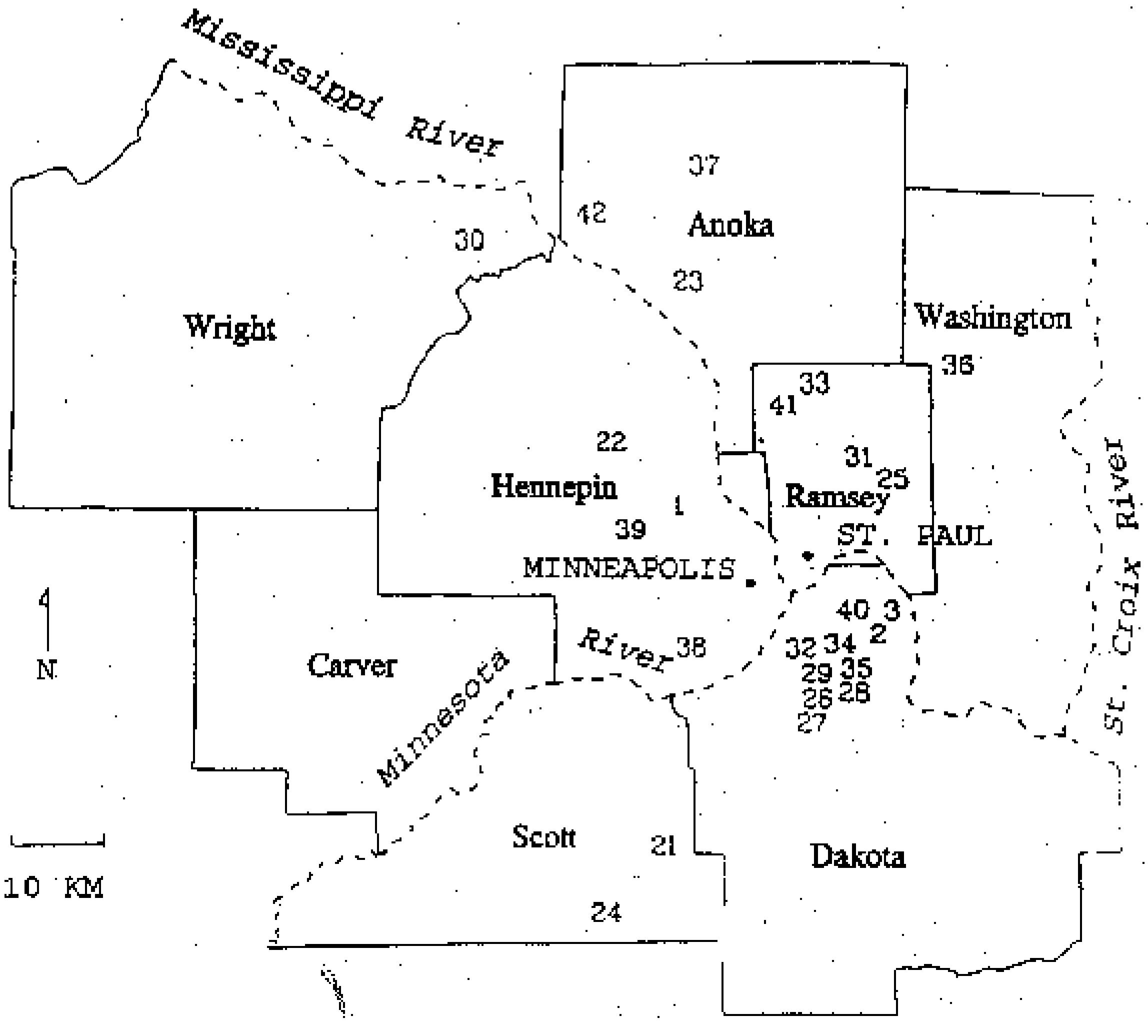

Figure 4-2. Map of eight-county Minneapolis/St. Paul, Minnesota, metropolitan area (adapted from Detenbeck, et al. 1992). Sites are listed by number and characterized in table 4-1.

Omernik (1986) defines the Twin Cities metropolitan area (TCMA) as part of the North Central Hardwood Forest ecoregion, with portions extending into the Western Cornbelt Plains. Topography consists of gently undulating, glaciated uplands dissected by the St. Croix, Minnesota, Rum, and Mississippi River valleys. The region is characterized by terminal moraines and glacial outwash with wetlands in areas of high water tables, in glacial kettle depressions, and along major rivers and associated tributaries (Ayers et al., 1985). Agricultural and urbanization pressures resulted in the filling or draining of many wetlands, and by 1969 only half of the presettlement wetland area remained (Anderson and Craig, 1984). Wetlands now constitute about 7.6 percent of the region (Owens and Meyer, 1978).

4.3.1.2. Site Selection

The study design limited the selection process to those wetlands that could be sampled before, during, and after disturbance within the two growing seasons of the original study time frame (September 1988 to October 1990). The lack of legal access eliminated only four of 53 wetlands identified as suitable for the project. Investigators also eliminated wetland disturbances adjacent to the St. Croix, Minnesota, and Mississippi Rivers because of the slight chance of observing a measurable impact to the riparian wetlands of these large, lotic systems. Impacts on water quality status and function of large riverine wetlands are better handled through cumulative impact assessments than site-specific or population studies (e.g., Gosselink and Lee, 1989; Osborne and Wiley, 1988).

Investigators identified 31 wetlands for the study by surveying wetland fill 404 permit notices and by requesting information on additional disturbance activities (dredging, impoundment, draining, storm-water inputs) from the Minnesota Pollution Control Agency (MPCA), Minnesota Department of Natural Resources (MN DNR), county (drainage) ditch commissioners, and watershed management organizations.

4.3.2. Problem Formulation

4.3.2.1. Stressor Characteristics

Disturbance activities identified through surveys of area resource managers included wetland fill (16), impoundment or dredging (9), and diversion of storm water or pumped ground water into wetlands (14; table 4-1). Construction activity in the watershed was quantified after the fact, when monitoring demonstrated that severe sedimentation problems existed at some sites. Investigators calculated physical or hydrologic disturbance intensities for each site based on field observations, 404 permit notices, and topographic maps combined with land-use maps derived by classifying aerial photos (1:9600) taken before and after disturbance activities (table 4-2).

In cases of dredging or impoundment, the disturbance was defined as a step change in water depth, based on field observations and design criteria contained in permit notices. Investigators used Circular 39 (Shaw and Fredine, 1956) definitions to classify pre- and postdisturbance wetland types. The difference in wetland types before and after disturbance was used as a measure of the intensity of dredging or impoundment. For example, a change from a type 3 shallow marsh to a type 5 wetland pond would have an intensity value of +2. Nonriparian

Table 4-1. Characteristics of Wetland Disturbance Study Sites in the Minneapolis/St. Paul Metropolitan Area (adapted from Detenbeck et al., 1992)

No.	Site	Watershed Area (ha)	Wetland Type(s)[a]	Wetland Hyd. Class[b]	Surrounding Land Use[c]	Disturbance[d]
1	Colonial Pond	20	3 (4)	IS (FL)	U/R	IMP, DRN, FLL, DRG, STRM, GR, ER
2	Wetland 23: Hwy 3	31	3	INT	AG (U/R)	ER, FLL
	Wetland 19: Hwy 3		3	IS (INT)	U/R	ER, FLL, STRM
	Wetland 21: Hwy 3		3	IS	AG (U/R)	ER, FLL
	Wetland 22: Hwy 3		3	IS	UN (U/R)	ER, FLL
3	Wetland 24: Hwy 3	5	4	IS (INT)	AG (U/R)	ER, FLL
	Wetland 25: Hwy 3		2 (5)	IS (INT)	UN (U/R)	ER, FLL, DRG
21	Credit R. Marsh	2,656	2, 2/6, 3	INT, INT, FL	AG	DRN
22	Bass Lake Wetland	1,336	4, 4	INT, INT	U/R	ER, FLL, STRM
23	Coon Cr. Ditch	40	2, 5	INT, INT	UN, U/R	STRM, DRG
24	Bradshaw Marsh	8,864	3, 3/7	FL	AG	DRN, DRG
25	Maplewood	188	2, 5, 7	INT	U/R	STRM, ER
26	LP-48	8	4	IS	U/R	ER, STRM
27	LP-31	76	5	INT	U/R	ER, STRM
28	JP-68	19	3	IS (INT)	U/R	ER, STRM
29	JP-26	42	5 (4)	INT, INT	U/R	ER, STRM
	JP-26W	42	4	INT	U/R	ER, STRM

Table 4-1. Characteristics of Wetland Disturbance Study Sites in the Minneapolis/St. Paul Metropolitan Area (continued)

No.	Site	Watershed Area (ha)	Wetland Type(s)[a]	Wetland Hyd. Class[b]	Surrounding Land Use[c]	Disturbance[d]
30	Albertsville	217	3/4 (4)	IS (INT)	UN	FLL, IMP
31	Centerville	9	4 (5)	IS (INT)	UN	ER, DRG, GR
32	JP-5	220	2/3, 3/6 (3/4)	FL	UN (U/R)	STRM, DRG
33	Rice Cr.	14	2/3	IS (INT)	UN	FLL, STRM
34	JP-23	19	4	IS (INT)	UN (U/R)	ER, STRM
35	JP-24	13	3	IS (INT)	UN (U/R)	ER, STRM
36	Ramsey Co. Ditch x	1,236	1 (1,4)	FL	UN (U/R)	IMP, FLL
37	Cedar Cr. S.E.	16	3	IS	UN	ER, FLL
	Cedar Cr. N.E.	16,000	1/2	FL	UN	ER, FLL
38	Comma Lake		4	INT	UN	ER, FLL
39	Minnehaha		5, 1	INT	UN	FLL/DRG
40	JP-25	21	2/3 (4/5)	IS (INT)	U/R	ER, DRG, STRM
41	Moundsview	40	6/7 (4)	IS (INT)	UN (U/R)	FLL, DRG
42	Trott Brook	10,000	1/2	FL	UN	ER/FLL

[a]Wetland type according to Circular 39 definitions; ( ) = Postdisturbance status.
[b]Wetland hydrologic class: IS = isolated from surface water inputs and outputs, INT = receiving intermittent inputs/outputs from adjacent surface waters, FL = receiving continuous surface water flow-through.
[c]Predominant surrounding land use: AG = agricultural, U/R = urban/residential, UN = undeveloped land.
[d]Disturbance types: IMP = impoundment, DRN = drainage, FLL = fill, ER = erosion, STRM = storm water, DRG = dredging, GR = pumped ground water.

Table 4-2. Summary of Wetland Disturbance Intensities for Study Sites in the Minneapolis/St. Paul Metropolitan Area (adapted from Detenbeck et al., 1991a)[a]

No.	Site	Change in Watershed: Wetland Area	Change in Urban: Wetland Area	Change in Residential: Wetland Area	Construction: Wetland Area	Fill Area (ha)	Fraction Wetland Filled	Wetland Type (Depth) Change (1-5)
22	BASS:EW MW-4	0.26	0.06	0.05	0.14	0.17	0.01	
22	BASS:EE MW-4	0.26	0.06	0.05	0.14	0.17	0.01	
37	CEDAR MW-1/2	0.19	0	0	0	0.11	0.05	
37	CEDAR DS	0.19	0	0	0	0.11	0.05	
37	CEDAR MW-3	4.05	0	1.09	0	0.06	0.11	
31	CENTERVILLE MW-4	-462.96	0	0	120.37	0		1
31	CENTERVILLE MW-5	-462.96	0	0	120.37	0		1
1	COLONIAL:NW MW-4	7.59	0.67	0.67	5.31	2	0.71	
38	COMMA MW-4	0	0	0	0	0.01	0	
23	COON MW-2	7.83	1.04	-0.35	2.26	0		
23	COON MW-5	7.83	1.04	-0.35	2.26	0		
23	COON DS	7.83	1.04	-0.35	2.26	0		
33	RICE MW-2/3	1.41	0	0.9	0	0.69	0.18	0.5
33	RICE DS	1.41	0	0.9	0	0.69	0.18	
35	JP-24 MW-3	25.67	0	1.33	22.33	0		
40	JP-25 MW-2/3	65	0	4.5	57	0		2
34	JP-23 MW-4	0	0	-0.2	10.8	0		
29	JP-26 MW-5	4	16	40	24	0		-1
29	JP-26W MW-4	4	16	40	24	0		0

Table 4-2. Summary of Wetland Disturbance Intensities for Study Sites in the Minneapolis/St. Paul Metropolitan Area (continued)

No.	Site	Change in Watershed: Wetland Area	Change in Urban:Wetland Area	Change in Residential: Wetland Area	Construction: Wetland Area	Fill Area (ha)	Fraction Wetland Filled	Wetland Type (Depth) Change (1-5)
32	JP-5 ML	-30.09	-3.32	-7.52	0.92	0		
32	JP-5:N MW-2/3	-30.09	-3.32	-7.52	0.92	0		
32	JP-5:SE MW-3/4	-30.09	-3.32	-7.52	0.92	0		1
32	JP-5:SW MW-6/3	-30.09	-3.32	-7.52	0.92	0		
28	JP-68 MW-3	-0.29	0	0	1.43	0		
36	RAMSEY CO DITCH #4 MS	0.13	0	0.06	0.15	0.69	0.01	3
36	RAMSEY CO DITCH #4 DS	0.13	0	0.06	0.15	0.69	0.01	
27	LP-31 ML	0	0.13	0.13	0	0		
26	LP-48 MW-4	0	0.5	-0.5	0	0		
39	MINNEHAHA MS	0.01	0	0	0	0.02	0	
39	MINNEHAHA MW-5	0.01	0	0	0	0.02	0	0
25	MNDOT:NW MW-2	0	0.24	0.12	0.36	0		
25	MNDOT:SW MW-5	0	0.24	0.12	0.36	0		
41	MOUNDSVIEW MW-6/7	0.67	1.33	-0.07	0	0.53	0.09	2
30	ALBERTSVILLE MW-3/4	2.86	0.07	0.23	0	1.22	0.12	1
42	TROTT MW-1/2	0.01	0	0	0	0.23	0	
42	TROTT DS	0.01	0	0	0	0.23	0	
2	HWY3:WTLD 19 MW-3/2	1	0	0.33	1.67	0	0.00	1.5
2	HWY3:WTLD 21 MW-3/2	0.28	0	0	0.49	0.1	0.09	
2	HWY3:WTLD 22 MW-3/5	0.13	0	0.01	0.59	0.15	0.05	
2	HWY3:23E MW-3	7.7	0	0.74	2.46	0.53	0.3	1
2	HWY3:23EE MW-3	7.7	0	0.74	2.46	0.53	0.3	1
3	WHY3:24 MW-4	3.52	0	0.19	2.82	0.79	0.53	0

[a]NOTE: Changes in land use or watershed area are expressed as a change in the ratio of watershed area, urban area, or residential area to wetland area ratio. Construction activity is expressed as the ratio of area of disturbed earth in the watershed to wetland area. Wetland depth change is expressed on a scale of 1-5 based on changes in wetland depths corresponding to types 1-5 according to Circular 39 definitions, where type 6 or 7 wetlands are assigned a depth of 2.

shrub-scrub (type 6) and woody (type 7) wetlands typically have water table levels equivalent to type 2 wet meadows and were assigned a hydrology factor of 2 on the intensity scale.

Investigators measured the intensity of fill disturbances as fill area, the percentage of wetland area filled, and the distance from the sampling point to the nearest area of wetland filled. Public notices published as part of the U.S. ACOE 404(c) permit program provided information on area of fill. Wetland areas were obtained from 404(c) permit notices, watershed districts, or National Wetland Inventory (NWI) maps. Distance from sampling point to nearest filled area was calculated from permit notice site maps, topographic maps, or NWI maps.

Storm-water inputs to a wetland are related to the degree of urbanization in the watershed. Increases in impervious surface area and point-source storm-sewer inputs increase the volume of storm water entering a wetland. To quantify the increase in urbanization, watersheds were gridded into 0.25- to 16-hectare cells on 1:25,000 U.S. Geological Survey topographic maps (depending on watershed size). Using this map, investigators identified the number of cells classified as urban or residential before and after disturbance, based on an examination of aerial photos (Detenbeck et al., 1991a). The change in the ratio of urban and residential area in the watershed to the area of each study-site wetland was used as one indicator of storm-water disturbance intensity. Because the creation of storm-sewer systems can involve connecting previously isolated watersheds, the change in watershed/wetland area was calculated as an additional index of hydrologic disturbance.

Investigators also used the gridded map to quantify erosion inputs. Construction zones with surfaces of freshly disturbed bare earth have the largest erosion potential. Therefore, the ratio of construction area in each watershed to postdisturbance wetland area was calculated for each wetland site, based on an examination of aerial photos.

Stressor impacts are determined not only by the incidence and intensity of physical or hydrologic disturbances but also by the frequency and duration of stressors, incidence of multiple stressors including increased chemical loadings from watershed development, and time since initial disturbance (recovery period). Ecosystem response also depends on tolerances of existing species, which may be related to prior disturbance history, including both anthropogenic and natural (climatic) disturbance regimes. Moderating factors include season, antecedent wetland type, vegetation, watershed conditions, and the use of best management practices (e.g., preservation of vegetated [upland] buffer strips).

4.3.2.2. Ecosystem Potentially at Risk

Wetlands in this area can be classified by either water depth or predominant vegetation type (e.g., Shaw and Fredine, 1956; Cowardin et al., 1979). Most of the freshwater wetland types identified by Cowardin occur in the study area (Owens and Myer, 1978; Werth et al., 1977), although bogs are extremely rare. Some calcareous fens occur in Dakota and Scott Counties in the southern part of the TCMA and contain plant species listed as endangered, threatened, or species of concern in Minnesota (Eggers and Reed, 1987). While wetland vegetation communities have been inventoried for the TCMA (Owens and Myer, 1978; Werth et al., 1977), few faunal inventories are available. A number of amphibians and reptiles are found in the study region, including eastern newts (*Notophthalmus viridescens*), tiger salamanders (*Ambystoma tigrinum*), leopard frogs (*Rana pipiens*), striped chorus frogs (*Pseudacris triseriata*), green frogs (*Rana clamitans*), wood frogs (*Rana sylvatica*), spring peepers (*Hyla crucifer*), snapping turtles (*Chelydra serpentina*), painted turtles (*Chrysemys picta*), and the smooth green snake (*Opheodrys vernalis*) (Niering, 1985). In all, 35 of the animal (27) or plant (8) species listed as endangered, threatened, or of special concern within Minnesota are associated with wetland habitats (MN DNR, 1984); 18 of these species have ranges that overlap with the study region (Niering, 1985; see table 4-3).

Table 4-3. Endangered, Threatened, and Special Concern Species in the Upper Midwest That Are Associated With Wetland Habitats (derived from MN DNR, 1984, and Niering, 1985)

Scientific Name	Common Name
Podiceps auritus	Horned grebe
Pelecanus erythroorhynchos	American white pelican[b]
Botaurus lentiginosis	American bittern[a,b]
Buteo lineatus	Red-shouldered hawk[a]
Pandion haliaeteus	Osprey
Grus canadensis	Sandhill crane
Rallus elegans	King rail[a]
Coturnicops noveboracensis	Yellow rail
Gallinula chloropus	Common moorhen[a]
Phalaropis tricolor	Wilson's phalarope
Sterna forsteri	Forster's tern
Asio flammeus	Short-eared owl[a]
Ammospiza caudacutus	Sharp-tailed sparrow
Clemmys insculpta	Wood turtle[a]
Chelydra serpentina	Snapping turtle[a,b]
Crotalus horridus	Timber rattlesnake
Acris crepitans	Northern cricket frog
Rana catesbiana	Bullfrog[a]
Rana palustris	Pickerel frog[a]
Clossiana frigga saga (Staudinger)	Frigga fritillary
Epidemia dorca dorcas (W. Kirby)	Dorcas copper
Eribia disa mancinus (Doubleday and Hewitson)	Disa alpine
Oeneis jutta ascerta (Masters and Sorensen)	Jutta arctic
Proclossiana eunomia dawsonii (Barnes and McDunnough)	Bog fritillary

Table 4-3. Endangered, Threatened, and Special Concern Species in the Upper Midwest That Are Associated With Wetland Habitats (continued)

Scientific Name	Common Name
Notropis emilae (Hay)	Pugnose minnow
Polyodon spathula (Walbaum)	Paddlefish[a]
Scaphirhynchus platorynchus (Rafinesque)	Shovelnose sturgeon[a]
Arethusa bulbosa L.	Orchidaceae[a]
Cephalanthus occidentalis L.	Rubiaceae[a]
Decodon verticillatus (L.) Ell.	Lythraceae
Hydrocotyle americana L.	Apiaceae[a]
Pinguicula vulgaris L.	Lenibulariaceae[a]

[a]Occurring within study region according to range maps in Niering (1985).
[b]Observed at least once in study site(s).

Hydrologic classifications for wetlands in the study area include (a) isolated wetlands, with no inlets or outlets; (b) intermittent-flow wetlands, with inlets and outlets that flow only during snowmelt or major storm events; or (c) flow-through systems, with a fairly continuous movement of surface water in and out of the wetland. Distinct differences in water chemistry exist among these hydrologic wetland types in the TCMA, with higher nutrient, carbon, and conductivity levels in isolated wetlands; thus response may differ by wetland type (Detenbeck et al., 1991a). Therefore, impact analyses should consider initial (predisturbance) wetland water quality as a reference condition.

4.3.2.3. Endpoint Selection

Surface water inputs and outputs to wetlands often are intermittent and cannot be rigorously quantified without intensive instrumentation and monitoring (e.g., Brown, 1985). Thus, mid-wetland water quality variables were chosen as the best set of measurement endpoints to indicate wetland condition and potential inputs to downgradient ground water or downstream surface waters.

Measurement endpoints were chosen as indicators of four components of mid-wetland water quality (transparency, trophic status, potential heavy metals toxicity, and redox status) and three components of downstream or downgradient surface water or ground-water quality (transparency, eutrophication, and potential toxicity to humans [nitrate] or aquatic biota [lead]). Within wetlands, reduced transparency from high dissolved organic carbon (DOC) or suspended solids will limit the growth of submerged macrophytes (Chambers and Kalff, 1985). Sedimentation can inhibit germination from seedbank sources (Galinato, 1985), which may already be depleted by dredge and fill activities. Qualitative records of dominant vegetation and plant cover at study-site wetlands suggested that recovery of submerged aquatics was delayed by $\geq$2 years following initial impacts (Detenbeck et al., 1992). Regional or local declines in submerged aquatic communities elsewhere have been attributed to eutrophication and reduced water clarity (Dennison et al., 1993). Phosphorus often is the limiting nutrient to primary producers in metropolitan area lakes, which are already predominantly mesotrophic or eutrophic (Metropolitan Council, 1981); thus, any increased loading to downstream lakes could be considered detrimental. Productivity of area wetlands can be either nitrogen- or phosphorus-limited; if it is nitrogen-limited, then increased nitrate loadings would also have an impact on wetlands.

Lead was chosen as a measurement endpoint because it is a common contaminant in urban environments. Lead levels are already elevated in metropolitan area lakes to levels exceeding water quality criteria (Metropolitan Council, 1981), and high lead levels are associated with urban storm- water runoff in this region (time-weighted annual average = 39 μg Pb/L; Johnston et al., 1990). Wetlands can efficiently retain particulate lead, thereby protecting downstream lakes but posing a potential risk to wetland biota (Detenbeck et al., 1991b).

Nitrate was chosen as a measurement endpoint because denitrification is an efficient and significant water quality improvement function associated with wetland ecosystems. A buildup of nitrate in urban wetlands would indicate a breakdown in normal wetland water quality function as well as a risk to users of downstream surface waters or ground water.

Measurement endpoints included changes in specific conductivity, temperature, dissolved oxygen, and ammonia because any of these might affect the suitability of wetland habitats. The analysis did not include measurements of sodium concentrations or ratios of monovalent to divalent cations, which would be expected to increase with an influx of road salt and could have deleterious effects on wetland phytoplankton or macrophyte vegetation (Wetzel, 1975). In particular, inputs of storm water to extremely dilute bogs or alkaline fens would be expected to effect ecologically significant changes in mid-wetland water quality (Rushton, 1991).

4.3.2.4. Conceptual Model

Elements of the conceptual model for this assessment are listed in figure 4-1, and the path diagram (figure 4-3) outlines the relationships between disturbance indices (stressors) and wetland water quality response. Construction activity is a potential source for sediment, phosphorus, and nitrate supplies in urban wetlands, while wetland fill area can serve as a source of sediment or phosphorus prior to revegetation. Construction activity also can increase loadings of dissolved

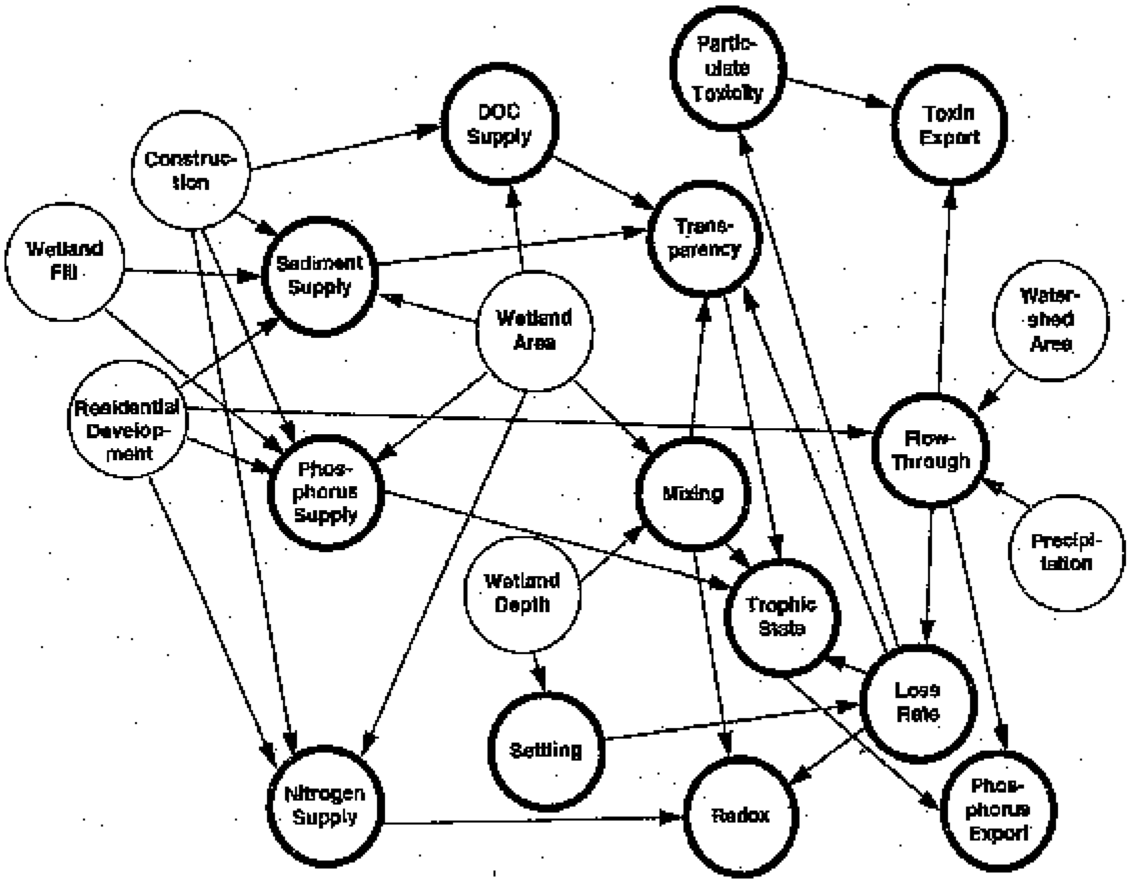

Figure 4-3. Path analysis diagram of cause/effect relationships. Exogenous factors (concepts) are indicated by thin-line circles, while endogenous factors are indicated by thick circles. Paths and directions of relationships are indicated by arrows. Four endogenous concepts (transparency, trophic status, particulate toxicity, and redox conditions) were chosen to represent components of mid-wetland water quality. See text for additional discussion.

organic carbon by disrupting soil structure and promoting degradation of soil organic matter. A large fraction of lead in urban runoff is in particulate form (particulate toxicity), thus lead loadings should be related to sediment inputs. Impacts of nutrient, organic carbon, sediment, and lead inputs are inversely proportional to wetland area; i.e., the same loadings will have a larger impact on a small wetland than a large wetland. Internal loading of sediment and phosphorus may increase as wetlands become shallower and resuspension increases, although development of emergent vegetation in the shallow marsh zone and of submerged vegetation may limit resuspension.

Losses of sediment, particulate-associated contaminants (lead), and nutrients (phosphorus) are controlled by hydraulic retention time (flow-through) and the time required for particles to settle out of suspension (settling), which is a function of particle size and wetland depth (Walker, 1987). Flow-through rates are dependent on runoff (a function of watershed area, percentage of impervious area, and precipitation) relative to wetland volume (surface area × depth).

Within each wetland, transparency is a function of dissolved organic carbon (color), turbidity (suspended solids), and to some extent, chlorophyll *a*. Chlorophyll *a* probably plays a lesser role in reducing water clarity in wetlands than in lakes, because algal production in the water column becomes inhibited by light limitation from suspended solids and water color. (Shading by floating algal mats would be an exception.) High turbidity, color, and trophic status (high chlorophyll *a*) within wetlands potentially limit the development or recovery of submerged macrophytes by limiting the depths at which sufficient light is available for growth (Chambers and Kalff, 1985; Dennison et al., 1993).

Redox status within the water column and surficial sediments will be reflected by levels of dissolved oxygen and by the proportion of dissolved inorganic nitrogen as ammonia. Under low dissolved oxygen conditions, relative levels of nitrate will decrease because of denitrification occurring in anaerobic sediments and because of inhibition of nitrification (conversion of ammonia to nitrate).

Comments on Problem Formulation

Strengths of the case study include:

- *General information on regional wetland types and species composition was summarized thoroughly.*

Comments on Problem Formulation (continued)

Limitations include:

- *The risk assessment was based on research that was primarily focused on wetland water quality function and attendant protection of downstream surface waters. Insufficient information was available to fully assess the impacts on wetland biota. An analysis of the biota found in specific study sites and an assessment of the relative sensitivity of different classes of biota would have strengthened the risk assessment. These data were not attainable given available resources. Research is under way to characterize the wetland macroinvertebrate communities that are affected by storm-water inputs in this region.*

- *Stressors and endpoints. Water quality might not be an appropriate endpoint to evaluate the impacts of disturbance to wetland ecosystems. For example, productivity is determined by the throughput, or turnover, of nutrients and can be affected without significant effects on standing stocks of free nutrients. Temperature is a relative factor; its impact depends on the system and its ground state. Also, the presence or absence of individual species is unlikely to be a sensitive indicator because of prior impact; however, changes in abundance might signal important changes.*

- *Wetland values. Wetlands are valuable for more reasons than serving as a buffer for downstream water quality. For example, wetlands provide habitat for migratory birds and ecotone species and help maintain ground-water levels. The case study should indicate how the stressors affect variables such as these. It also should be noted that partial fill is a loss of wetland habitat.*

General reviewer comment:

- *Although a path diagram is included (figure 4-3), a statistical path analysis could not be completed. The ecological literature tends to emphasize causality models that perform path analysis. If the data on wetlands are numerous enough or amenable to such analyses, a path analysis would be useful to decompose direct and indirect effects. The resulting diagram would be useful by showing linkages between dependent and independent variables. Rather than pattern hunting, statistics could be used to test specific hypotheses. The path analysis diagram could be used as a basis for the conceptual model.*

4.3.3. Analysis: Characterization of Exposure

4.3.3.1. Stressor Characterization

Among the study sites, dredging impacts ranged from minimal (no change in water depth class; e.g., Minnehaha site 39, type 5) to an increase in three depth units for site 36. In the latter case, a type 2 wetland was dredged to form a type 5 wetland pond. Impoundment in the absence of dredging was relatively rare, occurring at only two study sites, and resulted in a step change of only one unit in the scale of relative water depth (1-5).

Wetland fill area varied from 0.01 ha (Comma, site 38) to 2.0 ha (Colonial Pond, site 1), with percent wetland area filled ranging from less than 1 percent (Comma) to 71 percent (Colonial Pond). The greatest potential erosion impacts occurred at Centerville (site 31), with a ratio of 120 for construction zone to wetland area, indicating a high potential loading of sediment per unit surface area of wetland.

New storm-water inputs were common in urbanizing regions as the area of impervious surface increased and point-source storm sewers were built to divert storm water into wetlands. The potentially greatest storm-water impacts occurred at JP-26W (site 29), with an increase in the ratio of urban plus residential area to wetland area of 56. In two cases, ground water also was pumped into wetlands as a means to de-water adjacent construction sites. These inputs were temporary and sporadic and could not be quantified easily.

4.3.3.2. Ecosystem Characterization

Wetlands ranged in size from 0.01 ha to over 112 ha, and watersheds varied from 3.3 ha to 8,864 ha. Predominant land use in each watershed was classified as agricultural, urban or residential, or undeveloped or open space. Overall land use ranged from 0 to 92 percent agricultural, from 0 to 45 percent forested, from 0 to 48 percent urban, from 0 to 49 percent residential, from 1 to 58 percent water (lake plus marsh), from 0 to 70 percent construction area, and from 0 to 9 percent orchard in any given watershed. Average watershed slope varied from 1.4 percent for the Coon Creek watershed within the Anoka Sand Plain (site 23) to 13.2 percent for JP-25 (site 40) in the hilly terrain of Eagan. Soil erodability varied from an average K-factor of 0.16 (Coon Creek watershed, site 23) to an average K-factor of 0.33 in JP-68 (site 28) within Eagan.

Only a small proportion of the sites modified by physical or hydrological disturbance had buffers of undisturbed vegetation left surrounding the wetland. Typically, construction extended to the edge of or directly into the wetland. Only six wetlands had vegetated buffer zones left between the impact and the sampling point; these buffers ranged in width from 3 to 8 meters.

Effects of physical or hydrologic stressors will be moderated or exacerbated by climate, particularly the amount and temporal distribution of precipitation. Climate in the TCMA is continental, with mild, humid summers and relatively long, severe winters. Most rain comes in frontal storms or warm-weather convective storms, with May and June typically the wettest months and February the driest (Brown, 1984). Normal annual precipitation is 68.6 cm, including the water content of 111.8 cm average winter snowfall. Annual precipitation varied greatly during the study. In the drought year of 1989, annual precipitation was only 59.2 cm, while heavy summer rains brought the 1990 total to 83.9 cm (U.S. Weather Service, 1991).

Impacts on wetlands from physical and hydrologic disturbance depend in part on initial hydrologic and vegetation conditions at each site. Investigators classified the range of wetland types in the study area using the definitions in Circular 39 (Shaw and Fredine, 1956). Predominant vegetation in the wet meadows included reed canary grass (*Phalaris arundinacea*), smartweeds (*Polygonum spp.*), and stinging nettle (*Urtica dioica*). Cattail (*Typha spp.*) dominated both shallow and deep marshes, with sizable inclusions of softstem bulrush (*Scirpus validus*), giant reed grass (*Phragmites australis*), and arrowhead (*Sagittaria latifolia*). Various pondweeds (*Potamogeton spp.*), coontail (*Ceratophyllum demerusum*), and water milfoil (*Myriophyllum spp.*) were common among submersed vegetation, with lotus (*Nelumbo lutea*), yellow water lily (*Nuphar variegata*), white water lily (*Nymphaea odorata*), and duckweed (*Lemna spp.*) floating on the surface of wetland ponds. Invasions of purple loosestrife (*Lythrum salicaria*) were found in scattered areas. Typical shrubs in type 6 wetlands included red osier dogwood (*Cornus stolonifera*), speckled alder (*Alnus rugosa*), and numerous willows (*Salix spp.*). Of the species noted, seven were rated as moderately tolerant to turbidity and pollution by Kadlec and Wentz (1974), while only one species (*Potamogeton natans*) was rated as relatively intolerant. *Typha spp.* and *Phragmites australis*, common at many of the sites, are considered to be invasive species that often appear in disturbed areas.

4.3.3.3. Exposure Analysis

Frequency of impacts due to physical or hydrologic disturbance to wetlands in the TCMA was quantified by two related survey approaches. First, information on all impending physical or hydrologic disturbances to wetlands in the TCMA for the period of fall 1988 to fall 1990 was requested from area resource managers (see above). Second, all individual and nationwide U.S. ACOE 404 permits requiring predischarge notification received in 1988 and 1989 by the St. Paul District U.S. ACOE Office were reviewed for information on factors related to permit success (Taylor et al., 1992).

The frequency of disturbance regimes at sampling stations was tabulated by Circular 39 wetland type (figure 4-4). Most sites received multiple impacts. Almost all study sites (79 percent) were potentially affected by sedimentation from construction activity immediately surrounding the wetland or from physical modifications to existing wetlands. Nearly two-thirds of the study sites were partially filled or affected by storm-water or pumped ground-water inputs. Added water inputs were most common for wetland pond or deep marsh systems, while water level changes due to dredging or impoundment were most common for shallow marsh or wet meadow systems.

A total of 114 fill permits were reviewed, of which 86 (75 percent) were approved. Investigators identified 30 (35 percent) approved permits for which additional disturbances at the wetland site were anticipated, either as part of a construction project, mitigation action, or water level manipulation for waterfowl management. Eighteen (60 percent) of the additional disturbances

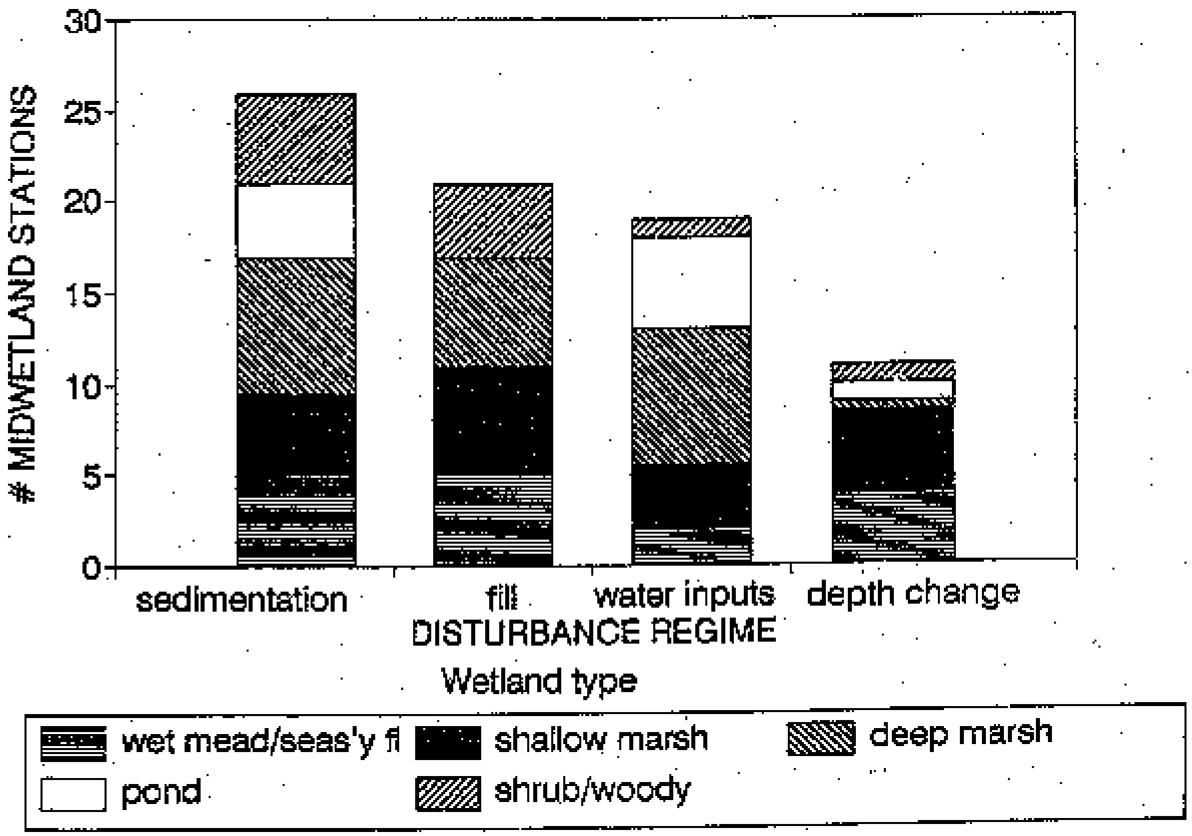

Figure 4-4. Frequency of disturbance regime by wetland type for study sites in Minneapolis/St. Paul metropolitan area (Detenbeck et al., 1991a). Physical or hydrologic disturbance regimes are categorized as sedimentation (i.e., erosion from construction activity or resuspension), partial fill activity, storm-water or pumped ground-water inputs, and water depth changes due to dredging or impoundment. Wetland sampling stations are categorized by Circular 39 classifications: wet meadow and seasonally flooded wetlands (types 1, 2), shallow marsh (type 3), deep marsh (type 4), wetland ponds (type 5), and scrub-shrub or woody wetlands (types 6, 7).

involved dredging open water areas, five (17 percent) involved new storm-water inputs, four (13 percent) involved dredging channels, three (10 percent) involved impoundment but no dredging, and three (10 percent) involved wetland drainage (two cases of temporary drainage).

Most of the wetland sites identified in this study received more than one physical or hydrologic disturbance, and many sites obviously had been affected by past alterations. Without a longer term record, however, it was not possible to determine the frequency of disturbance to wetlands in the TCMA over time. The season during which the physical activity creating physical or hydrologic disturbances ends is probably the most critical aspect of timing that will affect wetland recovery. At 34 (72 percent) of the 47 mid-wetland stations monitored, the physical activity producing the wetland disturbance ended outside of the growing season, i.e., in the fall, over the winter, or during snowmelt.

It is clear that physical or hydrologic disturbances affect some wetlands in the TCMA more heavily than others. However, there is no evidence that fill permit success is significantly associated with wetland type, adjacency to large wetland complexes, adjacency to calcareous fens (which have special protection status in the state of Minnesota), or state-protected status (types 3, 4, and 5 wetlands; Taylor et al., 1992). Permits to fill wetlands immediately surrounded by industrial or commercial land or by open land (on the suburban fringe) are significantly more likely to be approved than those for wetlands immediately surrounded by residential, mixed residential, or agricultural land use (Taylor et al., 1992). Storm water-related disturbances to wetlands are prevalent in the Eagan area (northern Dakota County), which has a relatively steep topography and a rapid rate of growth through residential development (figure 4-1).

An exact percentage of the area of wetland resources in the TCMA affected annually by physical or hydrologic disturbance cannot be easily quantified until automated data are available from NWI map digitization. Wetlands of 10 to 500 acres in size were partially catalogued in 1967 by MN DNR for fish and wildlife management (MN DNR, 1967). According to their records, approximately 745 type 2, 3, 4, or 5 wetlands were found in the TCMA. No quantitative inventory of smaller wetlands is available. If 86 wetlands are partially filled in a 2-year period, 30 of which experience physical or hydrologic disturbances, this represents an incidence of approximately 11.5 percent of wetlands affected by partial fill and 4 percent of wetlands affected by additional physical or hydrologic disturbance over a 2-year period. This obviously is an overestimate, however, because many of the wetlands affected by fill and related disturbances are much smaller than 10 acres in size, and this fraction of the wetland resource has not been well quantified in the TCMA.

Total wetland losses in Minnesota resulting from 404 permits (individual, general, and nationwide) equaled approximately 1,196 acres out of a total of 5.02 million acres, or 0.024 percent per year in 1988-1989. During the same period, Minnesota also saw a gain due to mitigation activities of 1,135 acres, for a net loss rate of 0.0013 percent per year (MPCA, 1990). In comparison, wetland losses due to drainage in a 10-county area during 1974-1980 were estimated at 0.02 percent per year for wetland ponds, 0.6 percent per year for deep marshes, and 2.3 percent per year for shallow marshes (MPCA, 1990). Loss of specific wetland types as the result of conversions to other wetland types has not been quantified for this region of the country. Nationwide, 0.1 percent and 1.3 percent of forested palustrine wetland area (swamps) have been lost by conversion to nonvegetated wetlands (ponds) and marshes, respectively, while 0.2 percent of marshes has been lost to conversion to ponds (Dahl et al., 1991).

4.3.3.4. Exposure Profile

The most commonly recorded physical or hydrologic disturbances to wetlands in the TCMA are, in order of frequency, sedimentation from excessive erosion, wetland fill, deepening by dredging or impoundment, and storm-water impacts. Up to 11.5 percent of wetlands in the TCMA were permitted for partial or complete filling over a 2-year period, with up to 4 percent of all wetlands affected by additional physical or hydrologic disturbances. Wetlands on the suburban fringe or those surrounded by industrial or commercial land uses are most likely to be filled. Storm-water inputs are probably most common in areas of rapid residential growth and relatively steep topography, but the use of wetlands for storm-water management is not well documented on a regional basis. Most disturbances to wetlands occur or terminate during a period outside of the growing season, thus maximizing potential recovery time.

Comments on Characterization of Exposure

Strengths of the case study include:

- *Spatial and temporal variability in exposures is described.*
- *The causes of uncertainty in exposure estimates are documented.*

Limitations include:

- *A complete exposure profile for the TCMA wetlands would require that the potential resources affected be better quantified in terms of wetland number, area, and type. In addition, a more complete sample of physical and hydrologic disturbance frequency and intensity, particularly for partially regulated or nonregulated disturbances (drainage, impoundment, dredging, storm-water or pumped ground-water inputs) is needed.*
- *A more complete exposure profile also would include site-specific information on particular wetland populations and communities exposed to physical and hydrologic disturbance, as determined by the overlap of their temporal and spatial distributions. Some of this information will be available from an ongoing study of effects of storm-water and nonpoint-source pollution on wetland macroinvertebrate communities in the TCMA.*

4.3.4. Analysis: Characterization of Ecological Effects

4.3.4.1. Evaluation of Relevant Effects Data

Investigators judged the relevance of the impacts of disturbance on wetland water quality on the basis of (1) the statistical significance of effects and (2) the potential ecological significance of effects. The statistical significance of changes in water quality was tested both as a verification of cause-and-effect relationships and as a means of comparing water quality values against a reference (predisturbance) condition. Comparisons against reference conditions are appropriate when water quality varies regionally as a function of landscape or climatic conditions or when there is a high level of uncertainty associated with the magnitude of critical effect levels. For example, reference conditions by ecoregion have been used in deriving regional lake water quality standards for the State of Minnesota (Heiskary and Wilson, 1990). Water quality criteria provide critical effect levels, but these often are derived based on tests of nonwetland species and under testing conditions (high dissolved oxygen, low dissolved organic carbon, circumneutral pH) that are atypical of wetlands (Hagley and Taylor, 1991).

The realism inherent in field-scale manipulations or observations is accompanied by spatial (geographic) variability among study sites as well as temporal (climatic) variability between pre- and postdisturbance periods. Analyses of predisturbance wetland water quality identified wetland type, hydrologic class, contact with sediment (pore water vs. surface water), season (snowmelt vs. growing season), and surrounding land use as factors with significant contributions to variability in wetland water quality variables (Detenbeck et al., 1991a). Thus, paired before-and-after comparisons were used to factor out spatial variability among sites. Predisturbance conditions at each site served as a reference against which peak- or postdisturbance conditions were compared using a parametric multivariate analysis of variance (MANOVA). A nonparametric Kruskal-Wallis test was used when data could not be normalized with log transformations (Sokal and Rohlf, 1981). MANOVAs were used in place of paired t-tests to reduce the probability of Type II errors, which increases as the number of tests performed increases. Repeated analysis of variance measures by variable type would be an ideal test to use here to determine time to recovery because these tests would correct for possible carry-over effects (serial correlation), but the number of observations available without missing data for all of the time periods of interest was very low (n=8-9).

The disadvantage of a paired before-and-after comparison approach is that interannual climatic variability can bias changes between pre- and postdisturbance periods. Thus, only those water quality variables showing both (a) a significant change from predisturbance periods and (b) a significant difference in response among disturbance classes were considered to be affected by physical disturbances. Alternatively, one could employ paired before-and-after comparisons with sites distributed along a disturbance gradient, somewhat analogous to an analysis of covariance approach. This approach regresses the change in mid-wetland water quality at each site against indices of disturbance intensity. Thus the y-intercept represents the expected change in water quality due to interannual climate variability alone, while the slope of the regression represents the response to increasing disturbance intensity. A multiple regression approach can be used to factor out the effect of multiple disturbances as long as colinearity (correlation among dependent variables) is not a problem.

Evaluation of levels of water quality variables (or changes in water quality) associated with potential ecological effects was based on water quality criteria values (total lead, ammonia, total phosphorus, nitrate, dissolved oxygen, temperature, conductivity, and turbidity) or on critical effect levels derived from the literature (color, turbidity, total phosphorus, and conductivity). Individual states are still in the process of modifying narrative and numeric surface water quality criteria for application to wetlands. According to guidance provided to individual states by the EPA Office of Wetlands Protection, initial narrative and numeric water quality standards for wetlands should be developed or modified using existing information as much as possible, with a longer-term goal of developing biocriteria for wetlands (U.S. EPA, 1991). Relevant water quality standards associated with designated (protected) uses for surface waters in the State of Minnesota are listed in table 4-4.

Table 4-4. State of Minnesota Water Quality Criteria for Surface Waters by Designated Use (U.S. EPA, 1988c)

Water Quality Variable	Units	State of MN[a] or Other Numeric Water Quality Criteria
Total phosphorus	µg P/L	Recreation.[b] 40 µg P/L for North Central Hardwood Forest ecoregion
$NO_3 + NO_2$	mg N/L	Consumption. >10 mg N/L
NH_4	mg N/L	For NH_3-N. Fisheries & Recreation. A: >0.016 mg/L; B: >0.04 mg/L
Surficial dissolved oxygen	mg/L	Fisheries & Recreation. 2A: ≥7 mg/L at all times. 2B, 2C: ≥5 mg/L at all times
Surficial water temperature	deg C	Fisheries & Recreation. A: no material increase, 30°C max. 8B, C: no increase in monthly avg. of max. daily temp. >1.7°C in lakes, 35°C max.
Total extractable lead	µg/L	4-day average. >1.3, 3.2, or 7.7 µg/L at 50, 100, 200 mg $CaCO_3$/L hardness
Specific conductivity	µmhos/cm	Agr. & Wildlife. A: >700 mg/L TDS[c]
Turbidity	NTU[d]	Fisheries & Recreation. A: >10; B, C: >25

[a]Depending on attainable use (Fisheries & Recreation or Agriculture & Wildlife). Class A = associated with salmonid fisheries, Class B = supporting cool- and warm-water sport or commercial fisheries and associated aquatic community, Class C = supporting indigenous fish and associated aquatic community.
[b]Based on attainable lake trophic state for North Central Hardwood Forest ecoregion.
[c]Approx. 1,094 µmhos/cm. TDS = total dissolved solids.
[d]NTU = nephelometric turbidity units.

Table 4-5. Water Quality Values Associated With Mean Light Requirements of 21.4 Percent Incident Radiation for Submerged Aquatic Vegetation in Northern Lakes (Chambers and Kalff, 1985)

Wetland Type	Depth Range, cm	K_d, m^{-1}	Secchi Depth, meters	Turbidity, FTU[a]	Color, PCU	Total P, µg/L
III	15-60	2.57	0.64	11.4	583	107
IV	60-120	1.28	1.29	5.2	268	N/A[b]
V	120-240	0.64	2.58	2.2	113	N/A[b]

[a]FTU = formazin turbidity units.
[b]Secchi depths outside of range of observations used in deriving equation.

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Changes in color, turbidity (suspended solids), and trophic state (total phosphorus [TP]) can be evaluated based on their effects on wetland transparency and on the potential for successful growth of submerged macrophyte communities. Relatively few data have been published on light requirements for submerged aquatic plants. Data have been compiled for sea grasses (Dennison et al., 1993) and for submerged macrophytes in the littoral zone of northern lakes (Chambers and Kalff, 1985). Chambers and Kalff report an average minimal light requirement for freshwater angiosperms in Canadian lakes corresponding to 21.4 ± 2.4 percent of surface light levels. Corresponding color, turbidity, and chlorophyll *a* levels, which would reduce light at the bottom of a type III, type IV, or type V wetland to 21.4 percent of surface illumination, can be calculated (table 4-5).

Calculations were based on the following relationships:

Equation 4-1 (Wetzel, 1975):

$$K_d = \text{extinction coefficient}$$
$$= \ln (I_0/I_z) \times 1/z$$
$$= \ln (1/0.214) \times 1/z_{max,m}$$

where I_0 = incident radiation, I_z = radiation at depth z

Secchi depth = $1.65/K_d$ (Dennison et al., 1993)

Equation 4-2 (Brezonik, 1978):

1/S.D. = 0.106 + 0.128 (turbidity, nephelometric turbidity units [NTU]) + 0.0025 (color, platinum cobalt units [PCU])

Equation 4-3:

$\log_{10}$(Secchi depth, cm) = 2.07 − 0.13 $\log_{10}$(total P, µg/L)

(Derived from data for colored lakes with average depth <2.4 m, in Beaver and Crisman, 1991.)

Minnesota's standard of 40 µg P/L for TP in lakes within the North Central Hardwood Forest is lower than the estimated requirement of 107 µg P/L to maintain sensitive submerged aquatic macrophyte communities in type III wetlands. The lower standard is ecoregion based and is designed to minimize the frequency of nuisance algal blooms.

During predisturbance conditions, mean specific conductivity values for surface water (440 µmhos/cm) and ground water (610 µmhos/cm) were near the upper end of the range associated with freshwater vegetation in the glaciated prairie region. Stewart and Kantrud (1971) list a range of <40-500 µmhos/cm for normal climatic conditions and a range of <40-700 µmhos/cm for extreme (drought) conditions. Thus, 700 µmhos/cm was considered a threshold value for specific conductivity for these wetlands.

Investigators had to apply dissolved oxygen criteria derived for other surface waters to study area wetlands because of a lack of better literature values. These criteria, however, were probably overprotective since wetlands in the study area typically contain no fish or fish species extremely tolerant of low dissolved oxygen (e.g., common carp [*Cyprinus carpio*], fathead minnow [*Pimephales promelas*], and brook stickleback [*Culaea inconstans*]). However, investigators believed that the average level and diurnal fluctuations in both dissolved oxygen and temperature could be critical in determining acceptable spawning habitat or refugia for amphibians.

4.3.4.2. Ecosystem Response Analyses

Investigators could not use MANOVA to test categorical effects with the full complement of study sites because data matrices were complete for a subset of sites; the power of these tests was more limited than for regression analyses. However, MANOVAs did demonstrate a significant fivefold increase in soluble reactive phosphorus (SRP) and a threefold increase in dissolved phosphorus (DP) at the peak of storm-sewer disturbance activities (after storm sewers were connected and during watershed construction activity; table 4-6). Threefold increases in SRP and DP were still evident in storm water-impacted sites at 6 to 12 months and at 12 to 24 months following peak disturbance. Nitrate levels were strongly elevated, fortyfold in dredged or impounded sites and eightfold in storm water-impacted sites during the peak of disturbance activity, but no significant (categorical) increases were observed during subsequent time intervals (table 4-6). All increases were significant at a probability level (α) of 0.05, some at a probability level of 0.001.

To assess further the long-term impacts of construction and residential development surrounding wetlands in urbanizing areas, nonparametric Kruskal-Wallis tests were used to compare different categories of wetlands. Wetlands in nondeveloping watersheds experienced declines in dissolved nitrogen between predisturbance and recovery periods, while those wetlands in developing watersheds experienced no change or a slight increase in dissolved nitrogen. Construction activity in the watershed was associated with increased ln (total suspended solids [TSS]) within wetlands in the second year following disturbance (Detenbeck et al., 1992).

Comparison of water quality changes among wetlands with or without vegetated buffers in watersheds with or without construction activity showed significant effects ($p<0.05$) only with respect to the initial impact period at the peak or immediately following disturbance. Wetlands without vegetated buffers in watersheds with construction activity had greater SRP levels than either wetlands associated with construction activity but surrounded by vegetated buffers or wetlands in watersheds without new construction activity. There was no significant difference between peak or predisturbance SRP for wetlands in watersheds without new construction activity and those surrounded by vegetated buffers. Nitrate levels followed the same pattern as SRP levels (Detenbeck et al., 1992).

DP was least in wetlands with no construction activity in the surrounding watershed but did not show significant differences between buffered and nonbuffered wetlands. Dissolved nitrogen and water color were greater in nonbuffered wetlands than in watersheds without construction activity. Longer-term effects of buffers were not detected for growing season averages of water quality variables in the first year following disturbance ($p>0.05$).

Table 4-6. Summary of Results of MANOVAs Testing for Significant Difference in Water Quality Change Among Disturbance Classes for Each of Four Time Periods

Variable	Time Period[b]	N	Average ln Ratio (post/pre)[a] (geometric mean) (*back-transformed 95% CI*) Depth Change	Wetland Fill	Storm Water
Δln soluble reactive phosphorus	1	19	-0.038	-0.090	1.65[c] (5.2) (*1.7 - 16*)
Δln dissolved phosphorus	1	19	-0.37	-0.081	1.21[d] (3.4) (*1.6 - 7.2*)
Δln nitrate	1	19	3.68[d] (39.6) (*10.4 - 151*)	-0.30	2.02[d] (7.5) (*2.9 - 19.7*)
Δln dissolved phosphorus	3	16[e]	0.24	-0.32	1.15[c] (3.2) (*1.3 - 7.3*)
Δln soluble reactive phosphorus	4	14[e]	0.17	-0.44	1.09[c] (3.0) (*1.2 - 7.6*)
Δln dissolved phosphorus	4	14[e]	0.11	-0.48	1.15[c] (3.2) (*1.4 - 7.2*)
Δln dissolved phosphorus	4	21	0.16	-0.39	0.71[c] (2.0) (*1.1 - 4.8*)

[a]Differences in the change in water quality between disturbance classes were tested by Tukey's test to control for experimentwise error. Categories not significantly different from each other are indicated by a line. Only variables demonstrating a significant change in water quality and significant differences in response among disturbance regime categories are included here.

(Notes continued on next page)

[b]1 = Peak-disturbance vs. predisturbance.
2 = 0-6 months postdisturbance vs. predisturbance.
3 = 6-12 months postdisturbance vs. predisturbance.
4 = 12-24 months postdisturbance vs. predisturbance.
[c]Different from zero: $p<0.05$.
[d]Different from zero: $p<0.01$.
[e]Only data for which both dissolved and total constituents were available (surface water samples) were included in the analysis.

4.3.4.3. Analyses Relating Measurement and Assessment Endpoints

The impact of physical or hydrologic disturbance on mid-wetland water quality depends on the ecological significance of the observed magnitude of change. In addition, potential impacts on biota of downstream surface waters must be considered. The impact of changes on water quality variables for which numeric water quality criteria exist or threshold values have been derived can be evaluated by assessing the incidence of criteria or threshold value exceedance.

4.3.4.4. Stressor-Response Profile

Investigators used stepwise multiple regression analysis to assess the effects of the intensity of physical or hydrologic disturbances on mid-wetland water quality. The change in water quality between pre- and postdisturbance time periods was the dependent variable. Although numerous statistically significant relationships were found, this case study reports on a subset focusing on water quality variables for which threshold values were derived or criteria were available (table 4-7). Equations were reported in greater detail by Detenbeck and colleagues in earlier reports (1991a, 1992).

Storm-water inputs, construction, dredging or impoundment, wetland fill, and increases in watershed area or area of urban or residential land use had a significant effect on mid-wetland water quality. Construction, particularly within the buffer zone surrounding wetlands, was correlated with increased concentrations of suspended solids, total lead, and nitrate in wetlands during the first year following disturbance. Increased urbanization relative to wetland area tended to increase total nutrient levels and the fraction of nutrients associated with particulate matter. Particulate nitrogen and phosphorus tended to increase as the area of wetland fill increased. Storm-water inputs (quantified as an increase in impervious surface area) tended to decrease dissolved nutrient levels in wetlands, probably by decreasing the water retention time of the wetlands (Detenbeck et al., 1992; Brown, 1985). When predisturbance mid-wetland water quality data were compared among sites, wetlands in isolated basins had significantly higher nutrient, dissolved organic carbon, and color values than did wetlands with intermittent or continuous flow. In effect, connecting previously isolated wetlands with a storm-water sewer network can flush nutrients downstream. Deepening wetlands by impoundment or dredging tended to lessen some

Table 4-7. Equations Predicting Change in Mid-Wetland Water Quality[a] as a Function of Disturbance Intensity (adapted from Detenbeck et al., 1991a, 1992)

Time Period Comparison	n	Adj. r^2	Disturbance Regime	Dependent Variable	Independent Variable[b] X_1	Independent Variable[b] X_2	Equation
Spring, ln (post/pre)	6	0.70	Storm water	TP	URB/WTLD	WSHD/WTLD	$Y = -1.4 + 0.20\ X_1 - 0.07\ X_2$
Spring, ln (post/pre)	6	0.91	Storm water	Color	CONSTRN/WTLD	WSHD/WTLD	$Y = -1.3 + 0.09\ X_1 - 0.055\ X_2$
Spring, ln (post/pre)	5	0.80	Storm water	Total extr. Pb	ln CONSTRN/WTLD		$Y = -1.8 + 0.46\ X_1$
ln (during/pre)	88	0.82	Fill	SRP	TYPEDIFF	FILL	$Y = -1.8 - 3.7\ X_1 + 4.3\ X_2$
ln (during/pre)	7	0.54	Fill	Color	TYPEDIFF		$Y = -0.3 - 1.0\ X_1$
ln (post/pre)	9	0.78	Depth change	SRP	WSHD/WTLD	FILL/WTLD	$Y = 1.7 + 0.007\ X_1 - 12\ X_2$
ln (post/pre)	6	0.90	Depth change	TP	CONSTRN/WTLD	WSHD/WTLD	$Y = -0.6 + 0.08\ X_1 + 0.022\ X_2$
Spring, ln (post/pre)	7	0.91	Depth change	Color	TYPEDIFF	WSHD/WTLD	$Y = 0.6 -3.1\ X_1 + 0.0045\ X_2$
Spring, ln (post/pre)	7	0.42	Depth change	TSS	WSHD/WTLD		$Y = -0.2 + 0.008\ X_1$
ln (recov/pre)	15	0.47	All	SRP	RES/WTLD		$Y = -0.3 + 0.16\ X_1$
ln (recov/pre)	12	0.61	All	TP	U+R/WSHD	WSHD/WTLD	$Y = -0.2 + 6\ X_1 - 0.2\ X_2$

[a]Growing season average unless otherwise stated.
[b]Areas in hectares: WTLD = wetland area; WSHD = watershed area; FILL = hectare fill area; TYPEDIFF = change in wetland depth (1-5); RES = residential area in watershed; U+R = urban + residential area in watershed.

changes in mid-wetland water quality, probably by increasing retention time and sedimentation efficiency.

Regression analyses also were used to determine the effect of disturbance intensity on recovery during the second year following disturbance. By that time, neither area of wetland filled nor percentage of wetland filled nor change in wetland type (water depth) had a discernible effect on mid-wetland water quality. However, changes in watershed land use relative to either watershed area or wetland area produced long-term effects on wetland water quality. Increases in urban or residential land use were associated with increases in dissolved nutrients (SRP, DP, N, and DOC) and TP and decreases in surface dissolved oxygen. An increase in the percentage of watershed developed (percentage urban and residential) was associated with a long-term increase in TP and decrease in surface water temperature. Construction activity in the watershed was correlated with decreased SRP and increased levels of suspended solids. As relative watershed area (and flushing rate) increased, dissolved N and P and TP tended to decrease, offsetting some of the increase due to accelerated nutrient loading.

Comments on Characterization of Ecological Effects

Strengths of the case study include:

- *A statistical analysis of ecosystem responses was conducted, allowing estimates of response along a gradient of disturbance as well as categorical response. Uncertainties due to Type I errors can be quantified.*

Limitations include:

- *Expected impacts due to water quality changes were derived in part from criteria developed for surface waters other than wetlands.*
- *Habitat impacts were not measured directly.*
- *Only static endpoints were used; ecological processes were not measured.*

General reviewer comments:

- *EPA is developing biocriteria to address ecological effects, but these criteria are not yet available for wetlands.*
- *Indices of abiotic ecological quality, e.g., habitat destruction, should be linked to biota.*

4.3.5. Risk Characterization

4.3.5.1. Risk Estimation

Investigators did not detect significant changes in mid-wetland specific conductivity, temperature, dissolved oxygen, or ammonium levels between pre- and postdisturbance periods, nor were any significant differences in response noted among disturbance categories by MANOVA. Peak-, postdisturbance-, or recovery-period-specific conductivity levels exceeded the threshold value of 700 $\mu mhos \cdot cm^{-1}$ in three cases, and temperature levels exceeded the absolute criteria for Minnesota lakes (35°C) in one case (table 4-8).

Table 4-8. Mean and Range of Growing Season Mid-Wetland Water Quality Values Prior to and Following Disturbance

Water Quality Variable	Units	Growing Season Predisturbance Mean (range)	Growing Season Peak-Disturbance Mean (range)[a]	Growing Season 1st Yr Postdisturbance Mean (range)	Growing Season 2nd Yr Postdisturbance Mean (range)
Total phosphorus	µg P/L	593 (29-2,806)	F 690 (110-1,712)	167 (25-766)	212 (25-904)
			D 312 (42-2,426)	261 (36-2,733)	375 (46-1,639)
			S 182 (32-511)	218 (23-996)	157 (40-559)
			M 132 (45-292)	159 (64-385)	272
NO_3+NO_2	mg N/L	0.43 (0.01-7.03)	F 0.55 (0.03-1.41)	0.16 (0.01-0.90)	0.21 (0.01-0.99)
			D 0.19 (0.01-0.79)	0.13 (0.01-0.86)	0.07 (0.01-0.34)
			S 0.19 (0.01-1.25)	0.07 (0.01-0.31)	0.03 (0.01-0.06)
			M 0.09 (0.06-0.12)	0.17 (0.03-0.61)	0.01
NH_4	mg N/L	0.32 (0.01-2.61)	F 0.05 (0.01-0.13)	0.13 (0.01-1.93)	0.15 (0.01-0.98)
			D 0.11 (0.01-0.91)	0.14 (0.01-2.53)	0.17 (0.01-2.03)
			S 0.03 (0.01-0.05)	0.08 (0.01-0.60)	0.07 (0.01-0.15)
			M 0.38 (0.21-0.49)	0.92 (0.06-2.67)	0.05
Surficial dissolved oxygen	mg/L	7.1 (1.2-15.4)	F 9.1 (8.4-10.6)	9.0 (3.5-12.4)	7.9 (3.0-16.0)
			D 4.5 (0.6-8.8)	5.8 (0.6-14.6)	3.1 (0.6-7.5)
			S 9.5 (2.7-13.6)	7.6 (1.3-20)	7.4 (1.3-14.8)
			M 6.8 (5.0-9.6)	4.9 (2.5-7.6)	12.0
Surficial water temperature	° C	16.6 (9.5-25.5)	F 18.8 (14.5-24.0)	19.4 (8.0-29.5)	21.4 (7.0-34.0)
			D 20.4 (14.0-29.3)	18.8 (8.0-28.0)	22.1 (12.0-29.0)
			S 22.3 (16.2-36)	20.0 (11.0-29.0)	21.0 (12.0-33.0)
			M 17.1 (10.8-21)	17.7 (13.0-25.0)	22.0
Total extractable lead	µg/L	9 (surface) 34 (porewater)			
Specific conductivity	µmhos/cm	398 (107-928)	F 133 (80-188)	300 (132-850)	283 (70-504)
			D 327 (164-759)	330 (153-688)	260 (162-404)
			S 239 (99-465)	216 (126-481)	206 (162-277)
			M 1120 (1,097-1,153)	758 (299-1,460)	283
Color	PCU	150 (32-746)	F 232 (34-601)	96 (40-226)	104 (38-260)
			D 140 (41-342)	150 (38-448)	131 (61-468)
			S 99 (19-343)	91 (26-305)	77 (44-135)
			M 48 (17-100)	82 (5-176)	170
Turbidity	NTU	24 (2-98)	F 282 (26-735)	27 (1-84)	15.2 (1-62)
			D 26 (3-127)	14 (1-279)	26 (3-128)
			S 34 (2-201)	19 (1-80)	12 (3-28)
			M 63 (15-133)	40 (3-123)	34

[a]F = partially filled, D = depth change (usually increase), S = storm-water or pumped ground-water inputs, M = multiple.

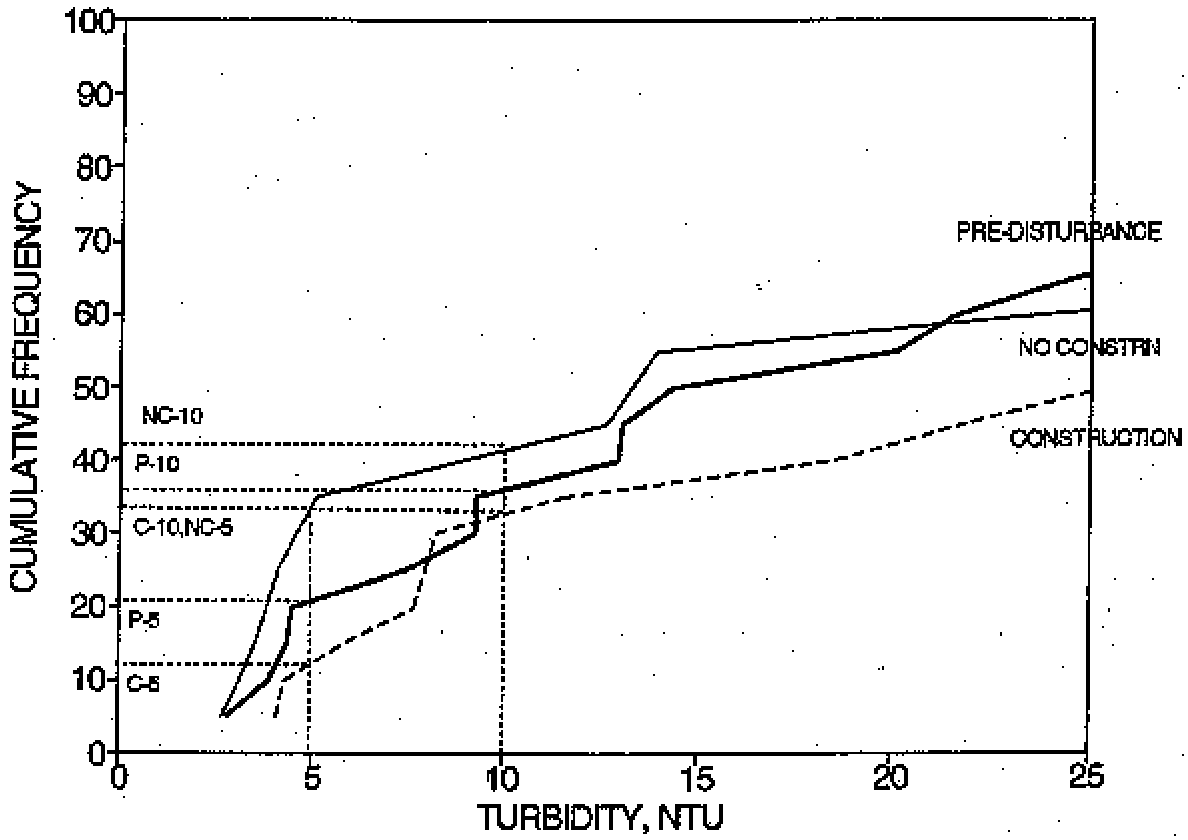

Figure 4-5a. Cumulative frequency distribution of average mid-wetland turbidity values over the growing season. Predisturbance turbidity distribution (P) is compared to peak-disturbance distributions for wetlands in watersheds with (C) and without (NC) construction activity. Proportions of the urban wetland population with turbidity at or below target levels of 5 or 10 NTU are indicated by the dotted lines.

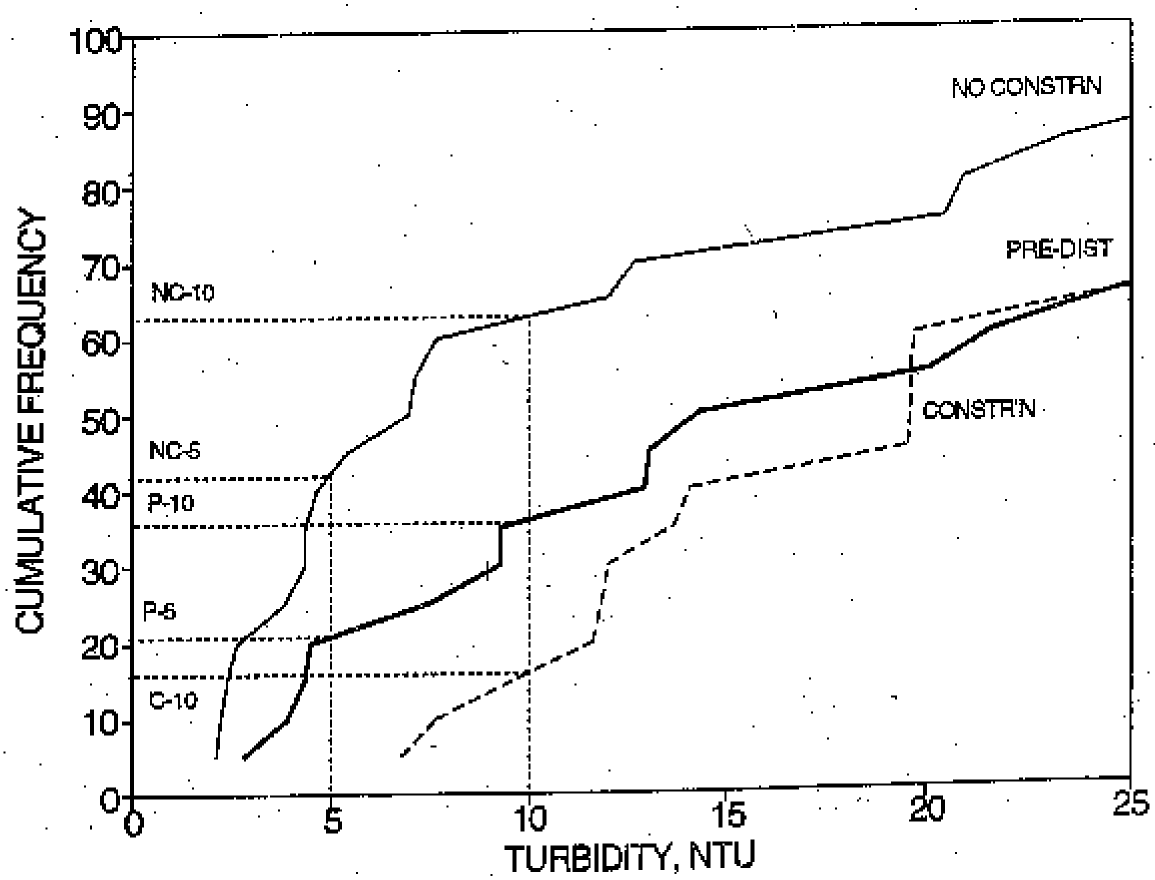

Figure 4-5b. Cumulative frequency distribution of average mid-wetland turbidity values over the growing season. Predisturbance turbidity distribution (P) is compared to first-year postdisturbance distributions for wetlands in watersheds with (C) and without (NC) construction activity. Proportions of the urban wetland population with turbidity at or below target levels of 5 or 10 NTU are indicated by the dotted lines.

The change in surface water temperature (as evidenced by the upward shift in minimum, mean, and maximum values between pre- and postdisturbance conditions) was >1.7°C for most cases, but differences in temperature increase were not noted among disturbance classes. This level of temperature variability may be natural for shallow wetland systems that are less resistant to temperature than are lakes.

Surficial dissolved oxygen levels were occasionally below the lower limit criteria for Class B and C waters for both predisturbance conditions (7 percent) and postdisturbance conditions (23 percent; table 4-8). Under the range of pH values measured for similar wetlands in the metropolitan area (pH 6-8; Detenbeck et al., 1991a) and the temperature range observed for these wetlands (7-36°C), approximately 2 to 11 percent of total ammonia plus ammonium would be present in the toxic (un-ionized) form (Thurston et al., 1974). Levels of total ammonium in wetlands during the predisturbance period were high enough to exceed Minnesota's water quality criteria for Class B and C fisheries and recreation surface waters (0.04 mg NH_3-N/L; U.S. EPA, 1988a) at approximately 60 percent of sites under the highest pH and temperature conditions observed. The proportion of sites at potential risk declined over the next 2 years to 5 to 15 percent following disturbance.

Mid-wetland nitrate levels increased significantly immediately following depth changes due to impoundment or dredging (39.6×) or immediately following storm-water inputs (7.5×). In no instance did nitrate levels exceed water quality criteria for drinking water standards; in general, nitrate levels were 1 to 3 orders of magnitude below the criteria of 10 mg N/L. However, if these wetlands are nitrogen-limited, a sevenfold to fortyfold increase in nitrate could be expected to stimulate productivity dramatically.

For water quality variables showing a significant categorical response to disturbance, the change in risk to wetland water quality or potential water quality function can be expressed as a frequency of criteria exceedance for pre- and postdisturbance populations. The majority of wetlands studied had predisturbance turbidity levels exceeding target levels for type III wetlands (64 percent >10 NTU) and type IV wetlands (21 percent >5 NTU; figure 4-5a). At the peak of disturbance, the frequency of sites exceeding target levels for type III wetlands decreased slightly for wetlands with no construction activity in the watershed (to 58 percent) and increased slightly for wetlands with construction activity in the watershed (to 66 percent; figure 4-5a). However, in the first year following disturbance, average turbidity levels exceeded target levels for type III wetlands for 37 percent of sites with no construction activity, and turbidity levels exceeded the target level of 10 NTU for 84 percent of sites exposed to construction activity (figure 4-5b).

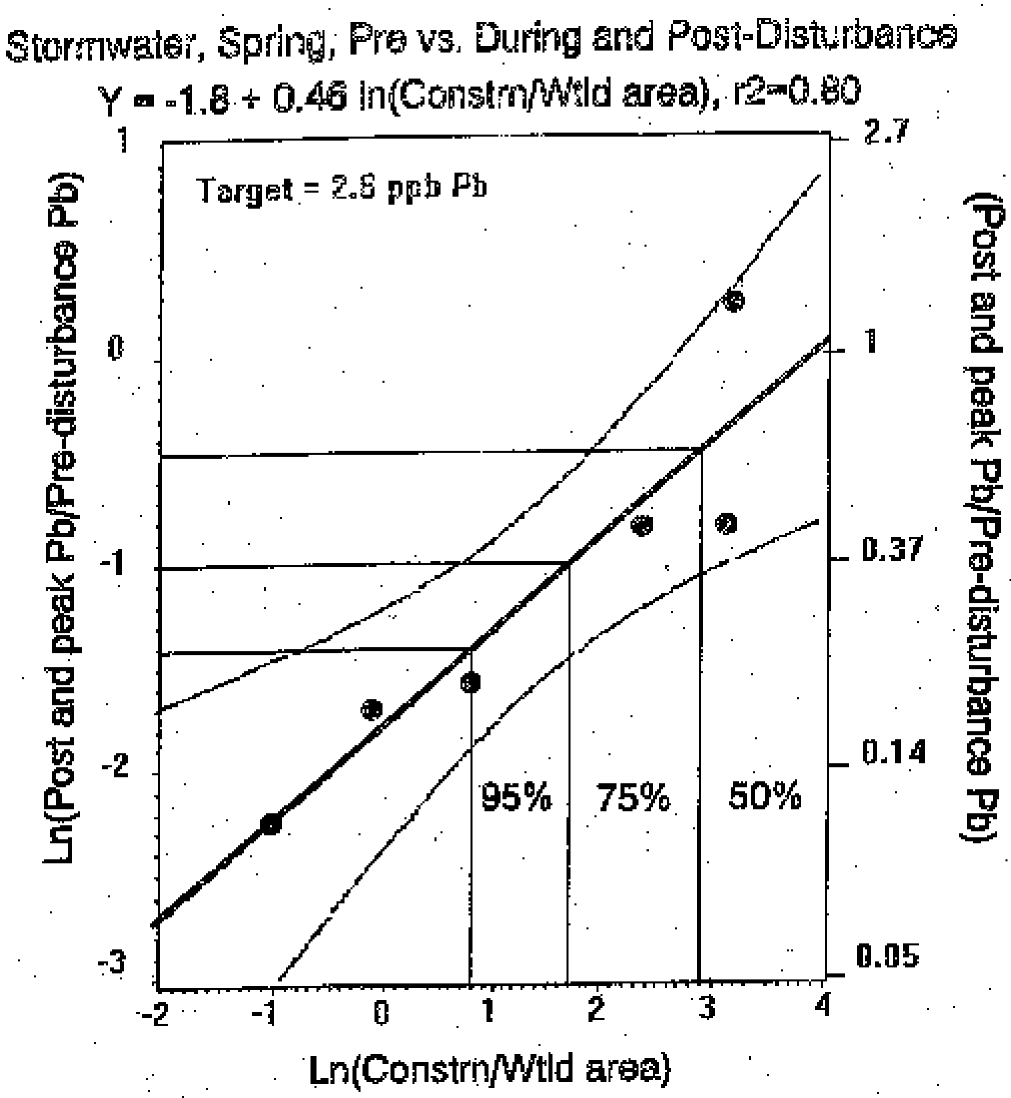

Figure 4-6. Regression line and 95 percent confidence interval for relationship between ln (construction/wetland area) and springtime ln (peak+postdisturbance Pb/predisturbance Pb) for urban wetlands affected by storm-water additions. Construction activity levels corresponding to protection of wetlands in the 95th percentile, 75th percentile, and 50th percentile (median) of the full predisturbance mid-wetland lead distribution are shown. Protection of mid-wetland water quality here is defined as a target level of ≤ 3.2 μg Pb/L.

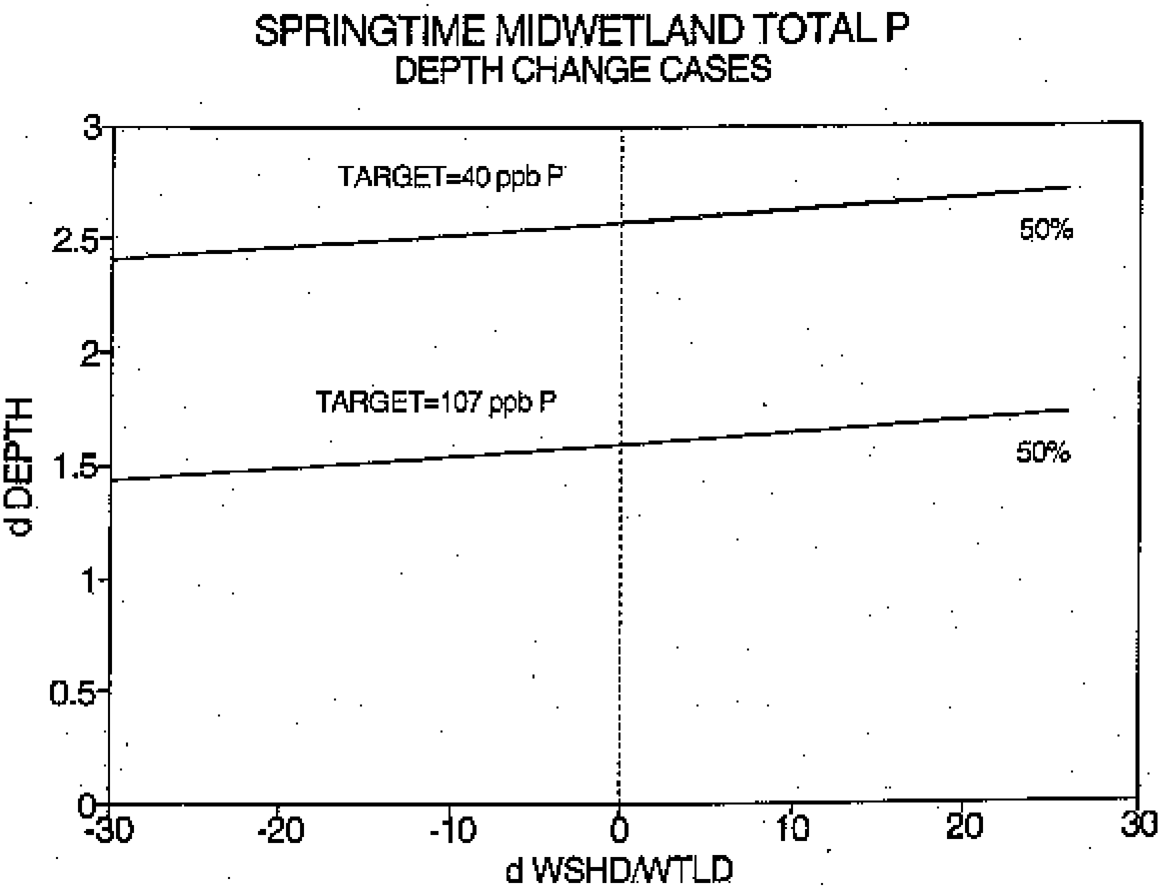

Figure 4-7a. Isopleths for mid-wetland TP threshold values of 40 μg P/L or 107 μg P/L; predictions for springtime mid-wetland TP, depth change cases. Isopleths define the combinations of two stressors predicted to yield the target value of mid-wetland TP based on the 25th percentile, 50th percentile (median), or 75th percentile of predisturbance TP distributions.

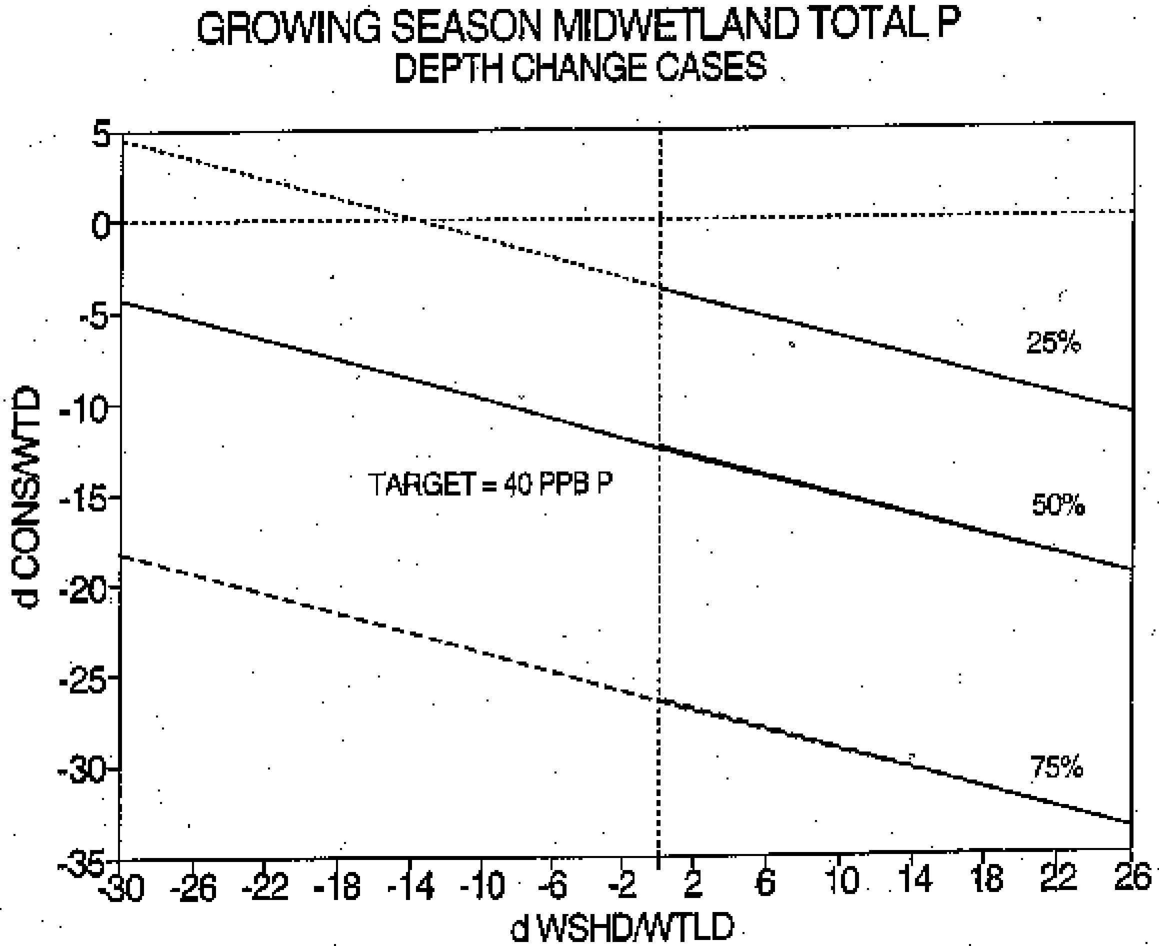

Figure 4-7b. Isopleths for mid-wetland TP threshold values of 40 μg P/L or 107 μg P/L; predictions for growing season mid-wetland TP, depth change cases, target level of 40 μg P/L. Isopleths define the combinations of two stressors predicted to yield the target value of mid-wetland TP based on the 25th percentile, 50th percentile (median), or 75th percentile of predisturbance TP distributions.

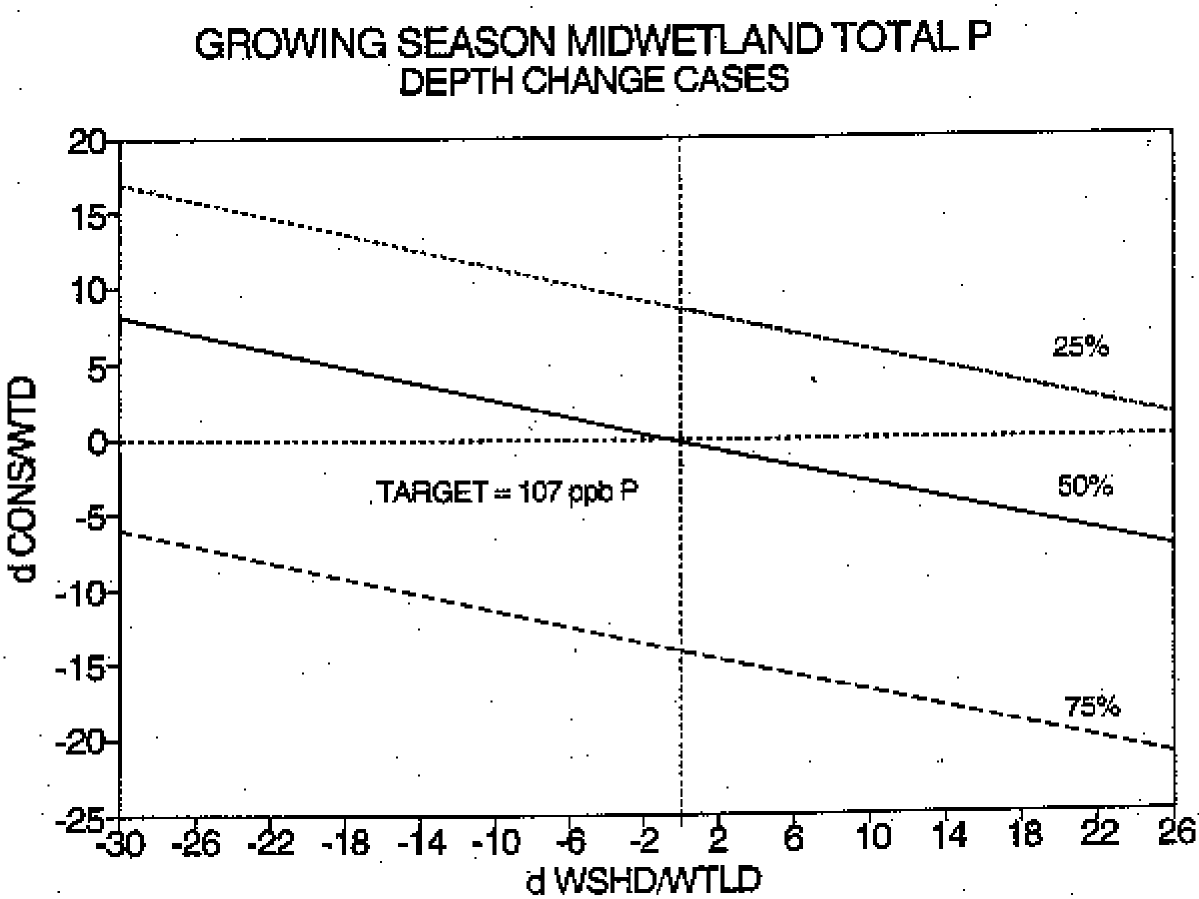

Figure 4-7c. Isopleths for mid-wetland TP threshold values of 40 μg P/L or 107 μg P/L; predictions for growing season mid-wetland TP, depth change cases, target level of 107 μg P/L. Isopleths define the combinations of two stressors predicted to yield the target value of mid-wetland TP based on the 25th percentile, 50th percentile (median), or 75th percentile of predisturbance TP distributions.

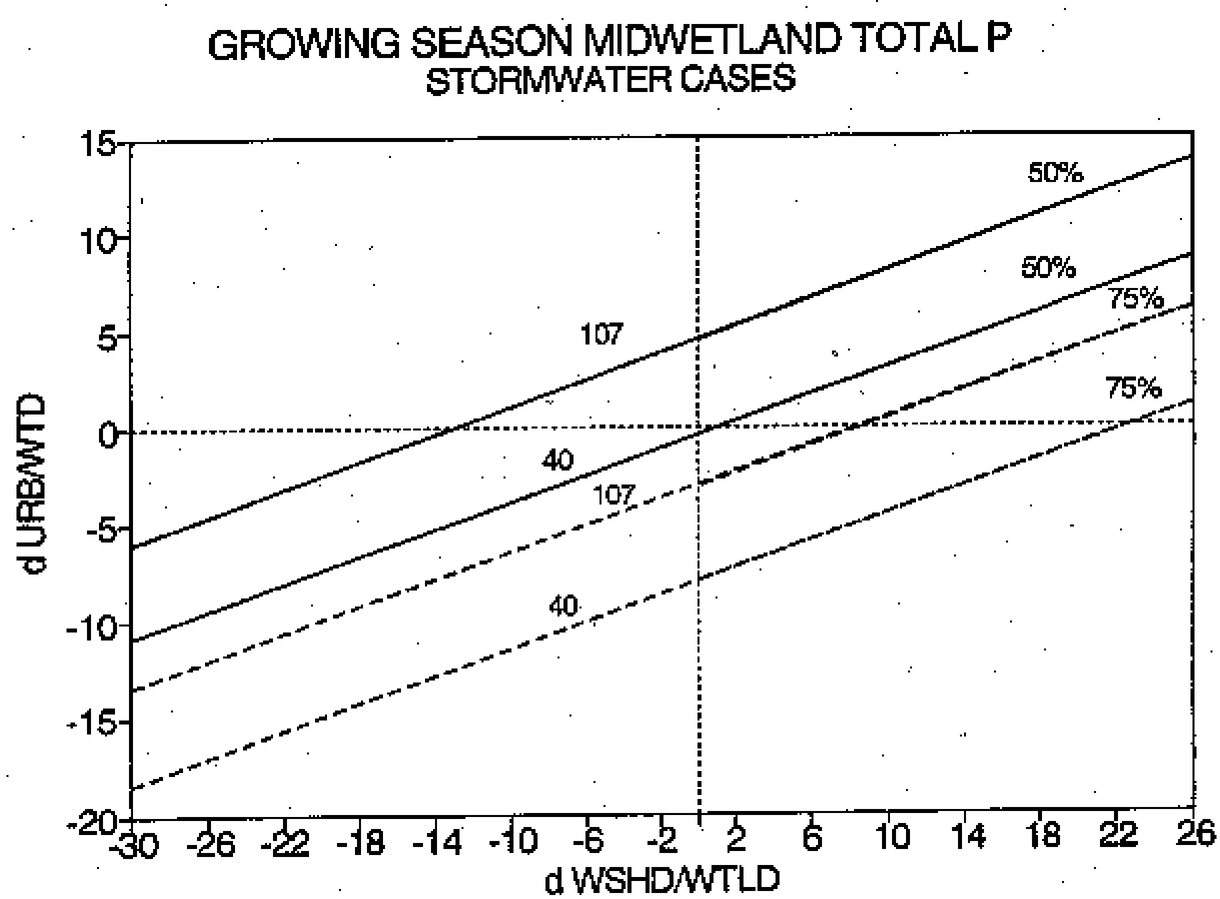

Figure 4-7d. Isopleths for mid-wetland TP threshold values of 40 μg P/L or 107 μg P/L; predictions for growing season mid-wetland TP, storm-water cases. Isopleths define the combinations of two stressors predicted to yield the target value of mid-wetland TP based on the 25th percentile, 50th percentile (median), or 75th percentile of predisturbance TP distributions.

Total extractable lead levels were quite high in the dry predisturbance period, possibly because of increased availability of lead following oxidation of lead sulfides or increased chelation by elevated dissolved organic carbon levels. The predisturbance average for surface water was 9 μg Pb/L, well above the criteria of 7.7 μg/L for chronic toxicity at a hardness level of 200 mg $CaCO_3$/L. Maximum mid-wetland lead levels declined between the pre- and postdisturbance periods, possibly due to increased precipitation and a lowering of redox levels. However, the magnitude of the inter-annual decrease in total lead decreased as a function of construction activity in the period immediately following storm-water inputs (figure 4-6).

The level of construction activity in the watershed associated with exceedance of the water quality criteria for chronic toxicity was calculated as a function of initial lead levels. Figure 4-6 shows the level of the ln (construction/wetland area) ratio associated with exceedance of the criteria for chronic toxicity (3.2 μg/L at 100 mg/L $CaCO_3$ hardness) corresponding to the 95th percentile, 75th percentile, and 50th percentile (median) values of predisturbance lead levels. Four of six sites had construction activity greater than that associated with criteria exceedance for the upper 5 percent of predisturbance values, three sites had levels associated with criteria exceedance for the upper 25 percent of predisturbance values, and two cases had construction activity (ln ratio >2.9, ratio >18.2) higher than that associated with exceedance for sites in the upper half of the predisturbance distribution. Similar predictions can be derived for other initial lead distributions. Predictions are based on mean response (ln [post/pre] Pb); the actual response is expected to fall within the 95 percent confidence interval for the regression.

Mid-wetland TP levels exceeded target levels derived to protect clarity of type III wetlands (107 ppb P) and criteria for Minnesota lakes in the North Central Hardwood Forest ecoregion (40 ppb P) in the majority of cases for predisturbance, peak-disturbance, postdisturbance, and recovery periods (70 to 80 percent >107 ppb P, 94 to 100 percent >40 ppb P). Based on results of regression analyses, potential increases in TP for storm water-impacted sites related to urbanization were offset by increased flushing rate related to increased watershed/wetland area ratios.

Investigators used regression equations for mid-wetland TP to predict stressor levels associated with criteria or threshold value exceedance for different percentiles of the population of predisturbance wetland conditions (figures 4-7a-d). For wetlands associated with the lower 50 percent of predisturbance TP levels, springtime TP levels were expected to exceed target levels of 40 ppb P and 107 ppb P for wetlands experiencing depth changes of <2.4 or <1.4 units, respectively (figure 4-7a). For the upper 75th percentile of sites, a target level of 40 ppb P could be achieved in cases of little or no construction activity (construction/wetland area <5) and a net *decrease* in watershed/wetland area (-13 to -30; figure 4-7b). The lower 50th percentile of cases were expected to remain below the target level of 107 ppb P only for cases of limited construction activity (ratio of 0 to 8) and no change or a decrease in watershed/wetland area (figure 4-7c). For wetlands affected by storm water, the lowest 50th percentile could achieve the target level of 40 ppb P following disturbance for increases in the urbanization ratio of up to 8, but only if relative watershed size (and flushing rate) was increased proportionately (figure 4-7d).

Color levels were elevated during predisturbance (drought) conditions corresponding to high dissolved organic carbon levels (Detenbeck et al., 1991a). However, only 2 percent of sites had color levels exceeding the target of 583 PCU for type III wetlands, approximately 20 percent

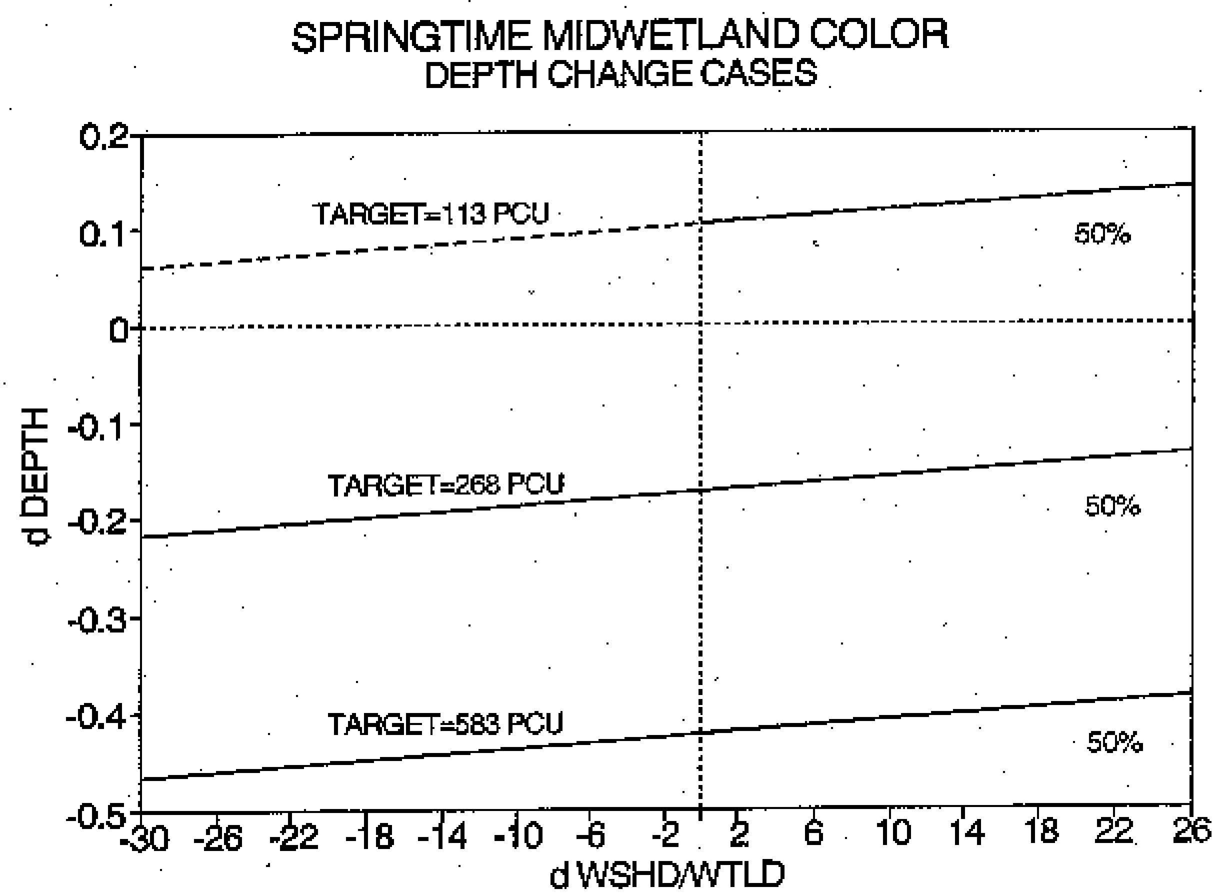

Figure 4-8. Isopleths for spring mid-wetland color target levels of 113 PCU, 268 PCU, and 583 PCU, based on predicted response of median predisturbance values and combinations of two stressors, depth change and change in watershed/wetland area ratios

of wetlands had color >268 (target for type IV wetlands), and 50 percent had color >113 PCU (target for type V wetlands) during the predisturbance period. Color alone probably is not limiting submerged macrophyte production in these systems. Maximum color levels for wetlands in fill and storm-water disturbance categories decreased following disturbance activities but increased between peak-disturbance and recovery periods for dredge or impoundment cases in response to increased watershed/wetland area ratios. Isopleth plots showing combinations of depth change and change in watershed/wetland for which target color levels could be achieved show that color levels are relatively insensitive to changing watershed/wetland area ratios and that the lowest target level for color is achievable for the lower 50th percentile given a small increase in depth (figure 4-8).

4.3.5.2. Uncertainty

Sources of uncertainty in this risk assessment include both qualitative errors (e.g., errors in assumptions) and quantitative errors (e.g., measurement or prediction errors). Table 4-9 lists the main sources of uncertainty in each phase of the risk assessment, along with an estimate of the magnitude of uncertainties.

Information gaps related to quantifying the total wetland resource in TCMA and the true frequencies of physical or hydrologic disturbances to wetlands contributed to overestimation and underestimation, respectively, of the true incidence of anthropogenic disturbance. Information gaps on direct habitat loss or conversion rates and lack of (tested) water quality criteria specific to wetlands limited the investigators' ability to create a balanced assessment of impacts to the full wetland ecosystem as compared with impacts to downstream surface waters.

Sources of uncertainty in the empirical field study on impacts and recovery included the interaction of effects of climatic variability between pre- and postdisturbance periods with effects of anthropogenic physical or hydrologic disturbance. By using the paired comparison regression approach, investigators were able to factor out potential additive effects of climatic differences between years but were not necessarily able to factor out interactive (e.g., multiplicative) effects. The design of the study would have been improved by including information from paired comparisons of undisturbed reference sites. Finally, the risk assessment could be improved by a separate field validation of regression predictions based on a separate set of study sites.

Table 4-9. Uncertainties Affecting Measurement of Risk to Urban Wetland Water Quality Status and Water Quality Improvement Function Related to Physical or Hydrologic Disturbance

Phase of Risk Assessment	Level/Measure of Uncertainty
Characterization of Exposure	
Total area, number of wetlands in metropolitan area	Unknown certainty; no quantitative updated inventory available
Incidence of physical or hydrologic disturbances to metropolitan area wetlands over time	Unknown, especially for unregulated activities
Intensity of physical disturbances to wetlands in metropolitan region	Range, distribution of measured values
Conversion factors for wetlands in metropolitan region	Unknown extrapolation error from nationwide trend analysis
Characterization of Ecological Effects	
Selection of threshold values or pertinent water quality criteria	Unknown certainty: (a) surface water quality criteria derived for clearwater lakes and streams, not wetlands; (b) water quality threshold values to protect transparency based on relationships derived for colored lakes and macrophyte depth distributions for relatively clearwater lakes
Estimate of relative risk due to habitat loss vs. water quality degradation	Direct effects of habitat loss or conversion on threatened or endangered species not measured
Measurement extrapolations	Temperature and dissolved oxygen min./max. values not recorded Loadings to downstream surface waters not directly measured
Precision/accuracy of water quality measurements	Relative error generally <10 percent
Probability of Type I error in identifying significant changes in water quality	$p \leq 0.05$
Stressor-response analysis	Type I error ≤ 0.05; uncertainty of predicted response indicated by regression r^2 values, 95 percent confidence intervals

4.3.5.3. Risk Description: Summary and Interpretation of Ecological Significance

Table 4-10 compares the risk to urban wetland water quality and water quality improvement function from physical or hydrologic disturbance to potential loss or conversions of wetland habitat. While neither dredging nor impoundment activity (water-depth change) caused many significant long-term changes in mid-wetland water quality, these activities probably had the greatest effect on wetland habitat. Wetland habitat is permanently removed by wetland fill activity and severely modified by dredging operations. Although emergent vegetation began to recover at disturbed wetland sites within 1 year following disturbance, the recovery of submerged vegetation appeared to be delayed by more than 2 years, especially where organic substrates had been removed (Detenbeck et al., 1992). Similarly, storm-water additions create a significant long-term shift in hydrologic regime, which may affect vegetation succession patterns and spawning habitat for amphibians.

Table 4-10. Summary of Risk to Urban Wetland Water Quality Status and Water Quality Improvement Function Assessed Against Loss or Conversion of Wetland Habitat

Activity	Percentage of Metropolitan Wetlands Affected[a]	Percentage of Metropolitan Wetland Area Affected	Nature/Probability of Direct Habitat Loss Through Destruction or Conversion	Metropolitan Area Wetlands Predicted to Exceed Water Quality Criteria or Threshold Values (%)	Potential for Water Quality Recovery
Fill	5.75%/yr	0.024%	Estimated mitigation of 94.9% of losses statewide	94-100% >40 µg P/L, 70-80% >107 µg P/L (historical impacts)	Recovery of all water quality parameters in <1 yr
Dredge/impoundment	4.8%/yr		100% type conversion for dredged/impounded sites Nationwide conversion rates: 0.1% type 7 to 5 1.3% type 7 to 3, 4 0.2% type 3, 4 to 5	94-100% >40 µg P/L, 70-80% >107 µg P/L (historical impacts)	Incr. NO_3 <1 yr
Storm water	1.0%/yr (new); cum. freq. approaching 100% in some areas		Permanent change in hydrologic regime	94-100% >40 µg P/L, 70-80% >107 µg P/L (historical impacts) 33% of storm water-impacted sites had construction activity high enough to produce spring Pb levels >3.2 µg/L on average	Incr. Pb, NO_3 <1 yr; potential release of Pb during drought Incr. turbidity >1 yr Incr. SRP, DP >2 yrs
Construction	4.5%/yr		Type conversion due to siltation at rate of 3%/yr for sites adjacent to construction activity	94-100% >40 µg P/L, 70-80% >107 µg P/L (historical impacts) 84% >10 NTU in first year following construction	Incr. TSS > 2 years
Drainage	0.3%/yr		100% habitat loss; historical losses reversing at rate ≤ 0.08%/yr of current area through restoration		

[a]Based on frequency of fill impacts and percentage of filled wetlands experiencing additional impacts.

Although the immediate effects of wetland fill on surface water quality are limited, the long-term cumulative effect of the loss or conversion of wetland area must be considered in determining risk to aquatic resources in this region. Earlier studies demonstrated a relationship between the extent of wetlands and low total lead or high color in downstream lakes, and between proximal wetlands and lowered trophic status in downstream lakes, or lowered suspended solids, fecal coliform, nitrate, and flow-weighted NH_4 or TP in streams of the TCMA region (Johnston et al., 1990; Detenbeck et al., 1991b, 1993).

Given the high level of total extractable lead in wetlands during the predisturbance period, any increase in lead would be considered detrimental to both wetland biota and biota of downstream surface waters. However, in the absence of disturbance activity, average total lead levels were predicted to decrease by 84 percent due to interannual climatic variation alone to levels just above detection limits. There is a high degree of uncertainty as to the actual impact of total extractable lead in wetland systems for two reasons. Surface water quality criteria were derived under standard testing conditions of low dissolved oxygen content, which may affect the availability of lead to biota. Second, much of the lead trapped in wetlands is associated with particulate matter, so that sediment concentrations and the potential for bioaccumulation need to be assessed (Stockdale, 1991).

Long-term categorical impacts on mid-wetland water quality were observed in response to construction activity and storm-water inputs (increased total and volatile suspended solids) or in response to residential development in the watershed (increased dissolved nitrogen). The proportion of wetlands with turbidity greater than identified thresholds for protection of submerged macrophyte communities increased over the first year following construction activity. The ecological significance of increased dissolved nitrogen in these systems is unknown at this point but could be very important if this change is an indicator of disruption of nitrogen cycling (Detenbeck et al., 1992).

Changes in land use (residential and urban development) and watershed area relative to wetland area were associated with statistically significant impacts on nutrients and water color in the first and second years following disturbance. However, it is clear that the trophic status of these wetlands is high due to prior loading. For fully or partially impounded wetlands, cumulative effects of wetland eutrophication may occur over time as loadings continue, but longer term studies are needed to assess these effects (Kadlec, 1985). Increased loadings of SRP or TP to wetlands converted from isolated potholes to components of storm water networks that experience intermittent or continuous flow probably create greater risks to downstream surface waters than to the wetlands themselves. The inverse relationship between watershed/wetland area and mid-wetland phosphorus concentrations for storm-water wetlands suggests that increased nutrient loads are being flushed downstream (Detenbeck et al., 1992). Given the high proportion of eutrophic and phosphorus-limited lakes in the TCMA, any additional inputs of phosphorus to downstream lakes are likely to be detrimental to these systems (Metropolitan Council, 1981).

Best management practices, such as the use of vegetated buffers, were only partially protective of mid-wetland water quality. Storm water represents a point-source input and is not filtered by vegetated zones surrounding wetlands. Vegetated buffers were associated with lower SRP and nitrate in wetlands with construction activity in the surrounding watershed, but this moderating effect was only temporary.

Comments on Risk Characterization

Strengths of the case study include:

- *Risk to wetland water quality is described both as a function of initial conditions (predisturbance water quality values) and as a function of the intensity of disturbance. Aspects of both temporal and spatial variability are addressed as they affect uncertainty estimates in risk analysis.*
- *A key feature of this case study is its predictive component: a stress-response tool developed as an empirical statistical model. Additional discussion is needed, however, regarding the representativeness of this data set for application to others.*

Limitations include:

- *Although quantitative estimates are provided for some elements of uncertainty (e.g., probability of Type I errors, experimental error values expressed as percent variance explained in regression analyses), most of the descriptions of uncertainty are qualitative. A rigorous quantitative analysis of overall uncertainty is not possible given the level of available information.*
- *A discussion of the larger issues associated with wetland assessment (e.g., landscape and wildlife aspects) is missing and could be included as a "lessons learned" section.*
- *Effects on organisms, especially mammals, are not discussed.*
- *The focus is on water quality impacts, while habitat destruction is glossed over.*
- *The potential forecasting use of the case study was not portrayed clearly and should be emphasized. Whether the study area wetlands are typical of those found in the area should be noted. Empirical models can be misused if differences between the study area and a new area are not understood.*

General reviewer comment:

- *With regard to mitigation, it is necessary to realize that virtually all wetlands were previously impacted, thus rendering it much less likely that perturbations of the kind reported here will result in further extinctions.*

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SECTION FIVE

ECOLOGICAL RISK ASSESSMENT CASE STUDY:

THE ROLE OF MONITORING IN ECOLOGICAL RISK ASSESSMENT: AN EMAP EXAMPLE

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7 **LIST OF ACRONYMS**

EMAP	Environmental Monitoring and Assessment Program
EPA	Environmental Protection Agency
ER-M	Effects Range-Median
GIS	geographic information system
HEP	Harbor Estuary Program
NEP	National Estuary Program
NOAA	National Oceanic and Atmospheric Administration
NPDES	National Pollution Discharge Elimination System
NRC	National Research Council
NS&T	National Status and Trends Program
ORD	Office of Research and Development
OTA	Office of Technology Assessment
R-EMAP	Regional EMAP

ABSTRACT

Using data collected from the Environmental Monitoring and Assessment Program's (EMAP's) Near Coastal program in the Virginian Biogeographic Province during July through September 1990-1991, this paper describes the role and specific contributions of monitoring data in the ecological risk assessment process. This case study suggests that EMAP monitoring data can:

- contribute to the problem formulation phase of an ecological risk assessment;
- characterize areal and spatial extent of ecological resources;
- identify regional resources potentially at risk (e.g., degraded benthos); and
- provide initial information on the role of exposure and habitat characteristics.

EMAP data were collected using a systematic probability-based sampling design that facilitates detection of spatially distributed patterns but does not estimate intra-annual variability or short-term episodic events. The EMAP information was then used to develop a conceptual model that described the areal extent of ecological resources at risk, their spatial distribution, and associated exposure and habitat information. The assessment endpoint was benthic community integrity. Resource condition, measured using a province-wide benthic index, was operationally defined in terms of one or more anthropogenic stressors. Currently, resource condition does not discriminate anthropogenic from natural physical stress.

In this case study, large estuaries exhibited the lowest areal extent of degraded benthos, 16±7 percent; low dissolved oxygen was the exposure indicator most closely associated with degradation. In small estuarine systems, 24±10 percent of the area exhibited degraded benthic condition, nearly half (48 percent) of which was associated with sediment toxicity. For large tidal rivers, 41±24 percent of the sampled area was degraded, and 45 percent of this degradation co-occurred with low dissolved oxygen. Co-occurrence of degradation and low dissolved oxygen was confined to the mouths of the Potomac and Rappahannock Rivers. Although these associations imply neither causality nor direct anthropogenic stress, they could, along with other evidence, be used to direct further study. In this regard, on a province basis more than half of the area of degraded benthos was not associated with any of the exposure indicators discussed.

Data on spatial distribution indicated that degradation of benthic resources occurred mainly in the upper Chesapeake Bay, the oligo-mesohaline portions of the five tidal river systems (e.g., Hudson-Raritan), and the associated small bays. These bays are areas of intense demographic pressure and extensive urban development.

Although useful in identifying regional areas of concern, EMAP province-scale data are not sufficient for conducting a complete risk assessment at the regional scale. Where local monitoring data are too heterogeneous (relative to spatial, temporal, and ecological scales and methodologies) to be usable in regional ecological risk assessments, investigators may need to acquire additional data through:

- an appropriately scaled monitoring program employing a random sampling design, such as the Regional EMAP [R-EMAP] program in EPA Region II;
- selection of the appropriate response, exposure, and habitat indicators to characterize the spatial extent of ecological problems and associated exposures; and
- incorporation of extant data (e.g., National Oceanic and Atmospheric Administration's [NOAA's] National Status and Trends [NS&T] Program, National Estuary Program [NEP], states, etc.).

Monitoring data alone cannot establish the causal relationships necessary to develop a complete analysis of ecological risk. Therefore, ecological risk assessments should include laboratory exposure-response information (e.g., ecotoxicity), effects of multiple stressors, and measures of contaminant bioavailability to provide evidence for postulating potential causes of risk to the region or to specific watersheds. Risks to specific watersheds can be examined initially by using geographic information system (GIS) and landscape methods that describe the spatial relationships and distribution of response, exposure, and habitat indicators (stressor-specific, whenever possible). This information can then be overlaid with landscape information on anthropogenic stressors and hydrologic features (e.g., transport and fate) in the surrounding watershed. Establishing causal relationships between sources and effects provides the basis for instituting appropriate control strategies. Ongoing local compliance (e.g., National Pollution Discharge Elimination System [NPDES], states, municipalities) and watershed assessment (e.g., R-EMAP, EMAP, NS&T, NEP) monitoring programs can evaluate the effectiveness of the control strategy.

5.1. RISK ASSESSMENT APPROACH

The U.S. Environmental Protection Agency's (EPA's) implementation of a risk-based assessment, monitoring, and decision-making strategy requires the integration of the Office of Research and Development's (ORD's) ecological risk assessment framework (U.S. EPA, 1992); research, monitoring, and assessment programs under ORD's Environmental Monitoring and Assessment Program (EMAP); and ORD's ecological risk assessment research programs. Successfully implementing a risk-based approach for decision making for adoption throughout EPA requires the integration of these three programs. The framework and process for conducting ecological risk assessment must not be separated from monitoring programs responsible for data acquisition and verification nor from research programs responsible for developing the needed methods and models. The combination of these programs provides the template for all ecological risk research, irrespective of specific programmatic applications, while ensuring that EPA can respond directly to the full spectrum of ecological risk assessment needs.

This case study illustrates the roles and contributions of EMAP's Near Coastal Program to the ecological risk assessment process as described by EPA's *Framework for Ecological Risk Assessment*. The case study also examines the use of monitoring data to identify potential problems for estuarine resources and the potential use of biogeographic province-scale information in regional assessments. Since EMAP and other monitoring programs typically are not designed to generate all the information required for a complete ecological risk assessment, this paper focuses specifically on the use of monitoring data (e.g., EMAP Virginian Biogeographic Province data from 1990 to 1991) in the problem formulation stage of the risk assessment process (figure 5-1). The areal extent and spatial patterns of ecological resources for the Virginian Province identify specific regional areas potentially at risk. The case study uses the Hudson-Raritan estuary as an example to illustrate the types of information needed for a complete ecological risk assessment.

5.2. STATUTORY AND REGULATORY BACKGROUND

The EPA, U.S. Congress, and private environmental organizations have long recognized the need to improve our ability to document the condition of our environment and specifically our ecological resources (National Research Council [NRC], 1990). Federal, state, and local agencies; waste dischargers; and researchers all conduct marine environmental monitoring. Five federal agencies conduct environmental quality monitoring activities in the coastal ocean. Each agency's programs focus on different spatial scales, ranging from effluent discharges from individual sources (e.g., EPA's National Pollutant Discharge and Elimination System [NPDES] Program) to measuring far-field, long-term effects of discharges from multiple sources (e.g., the National Oceanic and Atmospheric Administration's [NOAA's] National Status and Trends [NS&T] program, EPA's National Estuary Program [NEP]). However, these programs do not, either individually or taken together, constitute a comprehensive national status and trends monitoring program focused on contributing information for identifying the potential risks to coastal environmental resources (NRC, 1990). Congressional hearings on the Monitoring Improvement Act in 1984 (U.S. House of Representatives, 1984) concluded that, despite considerable expenditures on monitoring, federal agencies could assess neither the status of ecological resources nor the overall progress toward legally mandated goals of mitigating or preventing adverse ecological effects. In 1988, the EPA Science Advisory Board (U.S. EPA, 1988), affirming the existence of major gaps in environmental data and recognizing the broad base of support for better environmental monitoring, recommended that EPA initiate a program to monitor ecological status and trends of the nation's ecological resources. EMAP is EPA's response to these recommendations. This case study illustrates EMAP's contribution to the risk-based assessment framework that is the cornerstone of EPA's decision-making process.

5.3. CASE STUDY DESCRIPTION

This case study describes the role and contribution of monitoring data in the ecological risk assessment process. The EMAP response, exposure, and habitat indicator data presented in this case

PROBLEM FORMULATION

Stressors: Low dissolved oxygen levels, metals, and organic chemicals in sediments

Ecosystem(s) at Risk: Large and small estuaries and large tidal rivers in the Virginian Biogeographic Province

Ecological Components: Benthic macroinvertebrates

Endpoints: The assessment endpoint is benthic community integrity; measurement endpoints include the five indicators of benthic community structure that best distinguished between degraded and reference sites.

ANALYSIS and RISK CHARACTERIZATION

EMAP province-scale monitoring data alone cannot be used to complete the analysis and risk characterization phases of a risk assessment. However data needs and approaches for conducting such regional/watershed risk assessments are discussed.

Figure 5-1. Structure of assessment for EMAP Virginian Province

study were collected from the estuarine waters of the Virginian Biogeographic Province, which extends from Cape Cod, Massachusetts, to Cape Henry, Virginia, at the mouth of the Chesapeake Bay (figure 5-2).

Information from response, exposure, and habitat indicators constitutes the data acquisition component of ORD's *Framework for Ecological Risk Assessment* and contributes directly to the problem formulation stage of the risk assessment process. The monitoring data specifically contribute to the development of a conceptual model that delineates the spatial, temporal, and ecological boundaries of the problem; the specific ecosystems and ecological components potentially at risk; and the potential exposure pathways and co-occurrence with ecosystem attributes/resources of concern.

This case study analyzed data collected during 1990-1991 by determining the cumulative percent area (i.e., cumulative distribution function) for each ecological response and exposure indicator for the entire province and its component resource classes (large estuaries, small estuarine systems, and large tidal rivers). Because the EMAP sampling design is based upon a 4-year sampling cycle, the areal estimates based on 2 years of data reported in this case study for the response and exposure indicators must be viewed as examples of how the data can be used and should not be construed as the most complete or accurate reflection of the power of the EMAP sampling design. Since EMAP uses a probability-based design, the results from 2 years of sampling are likely representative of the remaining 2 years. However, the additional data will improve the estimates of central tendency, decrease uncertainty, and increase the power to detect change.

Analyses examined the associations between response and exposure indicators to explore the potential reasons for the observed changes in ecological condition. The areal extent of resource change that co-occurred with the exposure indicators was determined for the province as a whole and for each resource class. Information on exposure-response associations focused attention on specific regional areas, such as the Delaware Bay and the Hudson-Raritan estuary. For these areas, a full ecological risk assessment—a reiteration of problem formulation, the analysis of causal relationships, and the characterization of risks—can be conducted if the data warrant. Although these types of analyses are straightforward, their interpretation deserves discussion.

A typical assumption implicit in interpreting results such as these is that changes in resource status (e.g., degraded or subnominal condition) result from anthropogenic stress. One must view such interpretations with caution since these data are not designed to provide definitive information on causality or to separate anthropogenic from natural stressors. Rather they provide a "weight of evidence" approach, suggesting the direction for additional data acquisition and research. These data also are the basis for developing testable hypotheses to explain observations regarding the status of ecological resources. For example, low dissolved oxygen and physical alterations of habitats may not have anthropogenic origin in certain situations; therefore, they should not be associated with degradation, as defined by EMAP. For this reason, EMAP primarily seeks to determine the status of ecological resources. Although a useful and important part of the program, understanding the reasons for changes in status is secondary.

Although not explicitly part of this case study, a regional scale risk assessment could use both historical data (e.g., NEP, states, EPA Regions, academia) and new data (e.g., Regional-EMAP, EMAP) to characterize the magnitude and extent of the problem at the regional scale. In addition, changes in ecological resources can be coupled to specific stressors. Using geographic information system (GIS) and landscape methods, these stressors can be linked to potential sources associated with land-based activities. The overall effectiveness of control strategies applied to point and nonpoint sources could then be evaluated by both compliance (e.g., NPDES, states, dischargers) and long-term monitoring programs (e.g., R-EMAP, EMAP, states). This case study illustrates the application of a risk-based assessment and monitoring strategy that provides direct and indirect evidence for inferring causal associations between the observed ecological effects and specific stressors, thus enabling the manager to plan and evaluate remedial control strategy options.

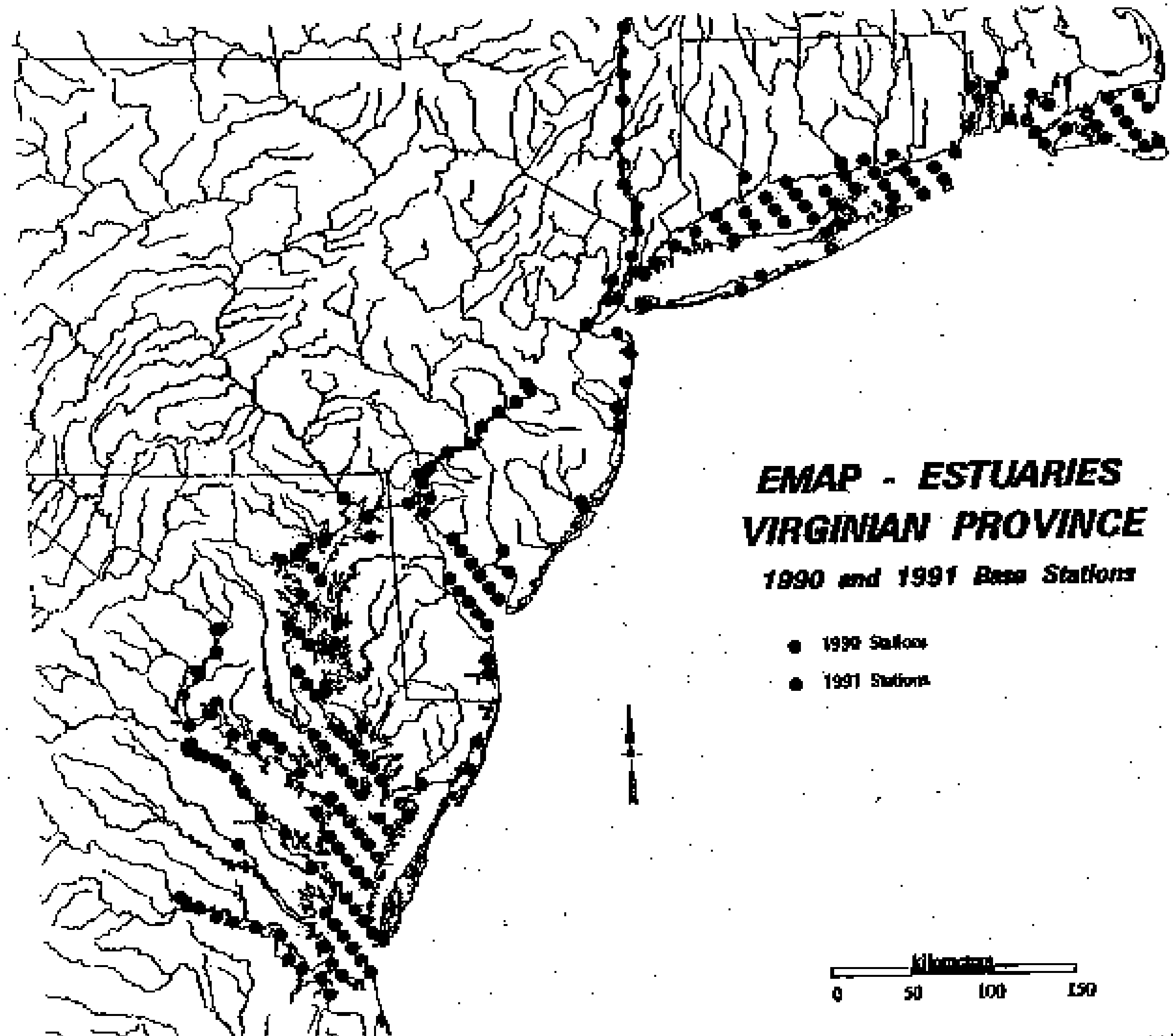

Figure 5-2. EMAP Virginian Province base stations for 1990-1991

5.3.1. Problem Formulation

5.3.1.1. Background

Problem formulation, the initial phase of the ecological risk assessment process, consists of the following components: stressor and ecological effects characterization, identification of ecosystems potentially at risk, selection of assessment and measurement endpoints, and development of a conceptual model (U.S. EPA, 1992). The conceptual model synthesizes the information in each of these components to describe the potential stressors and exposure pathways; their co-occurrence, direct and indirect links with specific ecosystems and assessment endpoints of concern; the spatial, temporal, and ecological boundaries of the risk assessment; and inferences as to potential causal associations between stressors and ecological effects. In this case study, the conceptual model describes (1) the areal extent of degraded benthic resources, (2) the areal extent of exposure to specific categories of stressors, (3) the relationship between the areal extent of degraded benthic resources and exposure to categories of stressors, (4) the relative importance of different stressors in each estuarine ecosystem, and (5) specific regional estuarine systems with degraded benthic resources that could become the subject of detailed regional risk assessments. The following sections describe the EMAP indicators and analyze and interpret data for 1990 and 1991 to provide information for the conceptual model.

5.3.1.2. Site Description

The data were collected from the estuarine waters of the Virginian Biogeographic Province, which extends from Cape Cod, Massachusetts, to Cape Henry, Virginia, at the mouth of the Chesapeake Bay. Covering approximately 23,573 km^2, the province includes several large estuarine systems (e.g., Chesapeake Bay, Delaware Bay, and Long Island Sound) as well as a substantial number of small estuarine systems and large tidal rivers (Holland, 1990). Both the Labrador Current and the Gulf Stream affect the Virginian Province, which has a continental/subtropical climate. Estuarine resources vary widely in size, shape, and ecological characteristics. Many estuaries, like Chesapeake Bay, are large, continuously distributed resources that consist of expansive regions with a broad variety of habitat types. Other estuaries consist of relatively discrete resources composed predominantly of one habitat type. For sampling design purposes, the estuarine waters of the Virginian Province were classified into three categories: large estuarine systems, large tidal rivers, and small estuarine systems.

5.3.1.3. Ecosystem Classification

Large estuarine systems are defined as systems having surface areas greater than 260 km^2 and aspect ratios (length/average width) less than 20. Application of these criteria to the Virginian Province resulted in the identification of 12 large estuarine systems with a total surface area of 16,096 km^2, or 70 percent of the province's estuarine area. Large tidal rivers were defined as systems having surface areas greater than 260 km^2 and aspect ratios greater than 20. These criteria resulted in the identification of five large tidal rivers—Hudson, Potomac, James, Delaware, and Rappahannock Rivers—with a total surface area of 2,840 km^2, or 13 percent of the total province area. Small estuarine systems were defined as systems having surface areas less than 260 km^2 but greater than or equal to 2.6 km^2. Application of these criteria to the Virginian Province resulted in the identification of 137 small estuarine systems with a total surface area of 4,279 km^2, or 17 percent of the province.

The classification process categorized estuaries into classes (strata) for which a common sampling design can be used. The process also ensured that selected components of estuarine resources were sampled sufficiently in different systems. Further, the classification process facilitated the synthesis and integration of data into assessments for evaluating the effectiveness of management actions (Holland, 1990).

5.3.1.4. Sampling Design

The EMAP sampling design provides unbiased estimates of the status and trends in indicators of ecological condition with known confidence. There are four essential features of the EMAP sampling design as applied to estuaries: regionalization, classification, statistical sampling, and index period. A regionalization scheme partitions the estuarine and coastal resources of the United States into geographical areas with similar ecological properties. The classification scheme defines certain populations of interest (e.g., large estuaries, small estuarine systems, etc.) within large geographical areas that are functionally similar and can be sampled using a common approach. The value of the EMAP sampling design is that it is both systematic in areal coverage yet probabilistic relative to the sampling strategy (Overton et al., 1991). This design, therefore, can determine areal extent (with confidence intervals) and the spatial patterns of response, exposure, and habitat indicators irrespective of the characteristics of their statistical distributions. The statistical sampling provides for the determination of unbiased estimates of the status and trends of the estuarine ecological resource classes. When fully implemented, EMAP will base its status assessments on data collected over a 4-year baseline (Holland, 1990). This multiyear cycle was chosen to dampen the year-to-year variability resulting from natural phenomena such as extremely dry or wet years and hurricanes. A consistent, probability-based sampling design is employed within each EMAP resource group to facilitate future integrated assessments among EMAP resource groups (e.g., estuaries, surface waters, forests).

Fully characterizing natural seasonal variability or assessing status for all seasons is beyond the scope of EMAP. Because intra-annual variability is thought generally to exceed interannual variability, an index period (July to September) was chosen to represent that portion of the year when the measured parameters are expected to show the maximum response to pollutant stress (Connell and Miller, 1984; Sprague, 1985; Mayer et al., 1989), dissolved oxygen concentrations are lowest (Holland et al., 1987; U.S. EPA, 1984; Officer et al., 1984), fauna and flora are most abundant, and within-season variability is expected to be minimal. This sampling design may fail to detect short-term, episodic events. However, persistent unexplained degradation identified by EMAP would certainly stimulate additional research in the area of concern. This approach is consistent with EMAP’s goals of determining the long-term status and trends of ecological resources, with the status and trends then being used as the basis for intensive site-specific research to understand the reasons for the observed problems.

Sampling sites in the large estuarine class were selected using a randomly placed systematic grid. The distance between the systematically spaced sampling points on the grid was approximately 18 km. The grid is an extension of the systematic grid proposed for use by all EMAP resource groups (Overton et al., 1991). For the Virginian Province, 54 sample sites were identified for the large estuaries for 1990, and 48 sites in 1991. Sampling sites were limited to waters >2 meters in depth; as a result of this limitation, investigators were unable to sample ~5 percent of the province area. In all cases, the entire large estuarine resource is sampled each year during the index period. A linear analogue of the above design was used for sampling site selection in the large tidal rivers. A systematic linear grid was used to define the spine of the five large tidal rivers in the Virginian Province. Randomly selected transects were placed along the spine of the river within sequential 25-km segments, starting at the mouth of the river and ending at the head of the tide. A total of 49 sample sites were selected for large tidal rivers in the Virginian Province in 1990 and 1991. The 137 small estuarine systems in the Virginian Province were randomly sampled from the entire list frame of small systems. They were ordered from north to south by combining adjacent estuaries into groups of four. One system was selected randomly from each group without replacement for each sampling year, yielding 62 sample sites for 1990 and 1991 in the Virginian Province. The location of the sample within each selected small system was randomly selected. Details of the design can be found in Holland (1990).

5.3.1.5. Ecological Indicators

EMAP defines and uses three types of ecological indicators: response, exposure, and habitat (Hunsaker and Carpenter, 1990). **Ecological response indicators** quantify the integrated response of ecological resources to individual or multiple stressors. Examples include measurements of the condition of individuals (e.g., frequency of tumors), populations (e.g., abundance, biomass), and communities (e.g., species composition, diversity). Because benthic communities play an important role in estuarine ecosystems (Holland et al., 1987, 1988; Rhoads et al., 1978; Pearson and Rosenberg, 1978; Sanders et al., 1980; Boesch and Rosenberg, 1981), this case study uses the condition of benthic assemblages as its only response indicator.

Characteristics of benthic assemblages have been used to measure and describe ecological status and trends of marine and estuarine environments for several decades (Sanders, 1956, 1960; Boesch, 1973; Pearson and Rosenberg, 1978; Holland et al., 1988). This literature has identified a diverse array of benthic assemblage attributes that can characterize ecological status and trends, including (1) measurements of biodiversity/species richness, (2) changes in species composition, (3) changes in the relative abundance or productivity of functional groups, (4) changes in relative abundance and productivity of "key" species, (5) changes in biomass, and (6) relative size of biota (Weisberg et al., 1993).

EMAP has operationally defined "degraded" or "subnominal" to classify the status of benthic resources. Three variables are used to characterize sites as degraded: sediment toxicity, sediment contaminants, and dissolved oxygen (Weisberg et al., 1993). Fifty-eight different attributes of benthic assemblages were evaluated and used to develop a "benthic index" to measure ecological status and trends in the Virginian Province. Of these, 28 benthic measurements differed significantly between degraded and reference sites and were candidates for the discriminant analyses that led to the development of a benthic index. While the operational definition of "degraded," as used by EMAP, assumes the presence of anthropogenic stress, alterations in benthic communities also can result from naturally occurring physical stresses and low dissolved oxygen. Since EMAP does not have an exposure indicator for eutrophication or physical stressors, the current benthic index may not always discriminate between natural and anthropogenic effects. This limitation suggests a need for additional exposure indicators. Finally, the term "degraded" also assumes some unique property or characteristic of benthic assemblages when, in fact, stressed communities reflect changes in successional status.

Using the 1990 data, five benthic measures (proportion of salinity-normalized expected number of species, number of amphipods, percent of total abundance as bivalves, number of capitellids, and average weight per individual polychaete) correctly differentiated reference sites from degraded sites with about 90 percent certainty (Weisberg et al., 1993). This version of the benthic index was specifically developed for the entire Virginian Province from 1990 data and may not be applicable outside the province or in other years. However, the important point is not the specific composition of the current index but rather the process of using discriminant analyses to identify combinations of candidate benthic measurements (measurement endpoints) that reliably distinguish between degraded and reference sites. This approach resulted in the development of a benthic index for 1991 data in the Louisianian Province that is analogous to the index for the Virginian Province (Summers et al., 1993).

Exposure indicators are physical, chemical, or biological measurements that quantify pollutant exposure, habitat degradation, or other causes of degraded ecological condition. Exposure indicators include direct measurements of contaminant or dissolved oxygen concentration in the water and sediments, contaminant concentrations in biological tissues, biomarkers, and acute toxicity of sediments. The Virginian Province study used three types of exposure indicators to infer changes observed in EMAP response indicators: metals and organic contaminant concentrations in sediments, sediment toxicity, and bottom dissolved oxygen. Clearly, these are not the only exposure indicators that are operative in estuarine systems and potentially responsible for ecological effects.

Metals and organic chemicals from freshwater inflows and from point and nonpoint sources concentrate in estuaries and accumulate in bottom sediments (Turekian, 1977; Forstner and Wittmann, 1981; Schubel and Carter, 1984; Nixon et al., 1986). These bottom sediments often are contaminated to the point that they represent a threat to humans and ecological components (Weaver, 1984; Office of

Technology Assessment [OTA], 1987; NRC, 1989). While the extent and magnitude of sediment contamination is only now becoming well described (NRC, 1989), it is a potentially important exposure indicator.

Whereas chemical measures of contaminant concentrations indicate the potential for ecological effects, sediment toxicity tests provide an indirect measure of contaminant bioavailability. A commonly used amphipod sediment toxicity test is well established and has been employed in a variety of monitoring and testing programs (Swartz, 1987, 1989; Chapman, 1988; Scott and Redmond, 1989; Scott et al., 1990).

Dissolved oxygen concentration is an important exposure indicator to both pelagic and benthic marine biota. Low dissolved oxygen is one of the more important factors contributing to fish and shellfish mortality in estuarine and coastal waters. Prolonged exposure to waters at less than 60 percent saturation can result in altered behavior, reduced growth, adverse reproductive effects, and mortality (Reish and Barnard, 1960; Vernberg, 1972). Excessive nutrient input can bring about low dissolved oxygen by stimulating phytoplankton blooms. Important as this indicator is to EMAP, its measurement presents special problems because of the wide diurnal and tidal fluctuations in concentrations. To address this problem, continuous and point sampling techniques currently are being evaluated (Holland, 1990).

Habitat indicators are physical, chemical, and biological measurements that provide information about the conditions (e.g., water depth, temperature, sediment characteristics, salinity) necessary to support ecological processes in the absence of pollutants. In estuaries, salinity and temperature are among the most dominant factors controlling the distribution of flora and fauna and the functioning of ecological processes (Remane and Schlieper, 1971). Sediment grain size has a role in regulating benthic community composition, while organic carbon affects the bioavailability of contaminants. Water depth itself can influence the temperature regime, salinity distribution, and dissolved oxygen concentration. These habitat variables are important for normalizing the responses of the response and exposure indicators and for defining subpopulations (e.g., fine vs. coarse-grained sediment, low vs. high salinity) for further analysis. In addition, these habitat indicators can be used to postclassify indicator data for a variety of analyses. For example, sediment toxicity data could be postclassified according to grain size or total organic carbon, both of which are known to affect contaminant bioavailability. Grain size also affects benthic assemblages in that benthos occupying sandy substrates are different from those dominated by silt-clay. EMAP's Virginian Province 1990 Demonstration Project Report presents discussions and examples of postclassification (Weisberg et al., 1993).

5.3.2. Conceptual Model Development

The goal of the problem formulation phase is the development of a conceptual model that identifies the potential relationships between valued ecosystem attributes (e.g., biotic integrity) and human or natural attributes, functions, or activities that are causes for concern (e.g., population density, deforestation, sea level rise, volcanic eruption). In the initial stages, problem formulation focuses on defining the two ends of a conceptual model: ecological responses in ecosystems potentially at risk and exposure to one or more stressors. The conceptual model identifies the potential exposure pathways by which stressors and ecosystem attributes may be connected to define the spatial, temporal, and ecological boundaries of the assessment and the ecosystems that are potentially at risk.

Figure 5-3 illustrates how monitoring data from the Virginian Province contributes to the components of problem formulation and the development of the conceptual model. As shown, the assessment endpoint is benthic community integrity; measurement endpoints include five specific benthic community metrics.

Analysis of areal extent for the status of each indicator represents only the area sampled during 1990-1991 and is not scaled to the total 4-year area. Presenting annual data provides a picture of year-to-year variability. In addition, unless otherwise noted, all data are presented as mean estimates within the bounds of the 95 percent confidence limits. Weisberg et al. (1993) provide details on these calculations.

5.3.2.1. Ecological Effects

This case study characterizes ecological effects by determining the areal distribution of degraded benthos using the assessment and measurement endpoints described above. Weisberg et al. (1993) describe the algorithm and rationale for calculating numerical values for the benthic index and the numerical cutpoint of <3.4 used to distinguish degraded from reference benthic condition. Cumulative distribution functions of benthic index values estimated the percent area of degraded benthos (Weisberg et al., 1993). Benthic index data from 1990 and 1991 were analyzed individually and then combined for the Virginian Province and for large estuaries, small estuarine systems, and tidal rivers (table 5-1).

- *Virginian Province*: The stations sampled in 1990 and 1991 represented 40 percent of the provincial area. Degraded benthic assemblages occurred in 19±6 percent of the province for the combined years and for each individual year (with slightly larger estimates of uncertainty).

- *Large Estuaries*: The stations sampled in 1990 and 1991 represented 40 percent of the large estuarine area. The study identified degraded benthic assemblages in 16±7 percent of the sampled area; there was little difference between years 1990 and 1991 (15±10 percent in 1990 vs. 17±10 percent in 1991).

- *Small Estuaries*: Thirty-nine percent of the area found in small estuarine systems was sampled in the 2 years. Of this area, 24±10 percent exhibited degraded benthos; again the difference between years was small (22±17 percent in 1990 vs. 25±16 percent in 1991).

- *Large Tidal Rivers*: The 2-year sampling accounted for 34 percent of the tidal river area in the province. Forty-one (±24) percent of the sampled area exhibited degraded benthos. The estimates of degraded condition showed large differences for the 2 years: 57±40 percent of the area in 1990 was degraded compared with 19±13 percent in 1991.

This case study used the benthic index to classify the areal extent of degraded benthic assemblages in the Virginian Province and its component resources classes. Figure 5-4 shows a pattern of increase in the percent area of degraded benthos across resource categories (1990-1991): 16 percent for the large estuaries, 24 percent for the small estuarine systems, and 41 percent for tidal rivers. Uncertainty estimates for areal extent of degraded benthos were within 6 percent for the province, 7 percent for large estuaries, 10 percent for small estuarine systems, and 24 percent for large tidal rivers.

Table 5-1. Summary of EMAP Response and Exposure Indicator Data for 1990-1991

	Estuarine Classes			
Indicators	Province (23,573 km^2)	Large (16,889 km^2)	Small (4,875 km^2)	Tidal (2,602 km^2)
Benthic index (1990-1991)				
Number of stations	206	96	61	49
Sampled area (km^2)	9,546	6,720	1,927	899
% Degraded area (B.I.<3.4)	19±6	16±7	24±10	41±24
Benthic index 1990				
Number of stations	105	48	32	25
Sampled area (km^2)	4,931	3,360	1,050	521
% Degraded area (B.I.<3.4)	19±9	15±10	22±17	57±40
Benthic index 1991				
Number of stations	101	48	29	24
Sampled area (km^2)	4,615	3,360	877	378
% Degraded area (B.I.<3.4)	19±8	17±10	25±16	19±13
Dissolved oxygen (1990-1991)				
Number of stations	198	94	59	45
Sampled area (km^2)	9,299	6,580	1,910	809
% Degraded area (D.O.<2.0 ppm)	6±4	5±4	<1.0	26±26
Dissolved oxygen (1990)				
Number of stations	97	46	30	21
Sampled area (km^2)	4,683	3,220	1,032	431
% Degraded area (D.O.<2.0 ppm)	7±6	6±7	<1.0	37±42
Dissolved oxygen (1991)				
Number of stations	101	48	29	24
Sampled area (km^2)	4,616	3,360	878	378
% Degraded area (D.O.<2.0 ppm)	4±4	4±5	1±2	15±27

Table 5-1. Summary of EMAP Response and Exposure Indicator Data for 1990-1991 (continued)

	Estuarine Classes			
Indicators	Province (23,573 km^2)	Large (16,889 km^2)	Small (4,875 km^2)	Tidal (2,602 km^2)
Sediment toxicity (1990-1991)				
Number of stations	172	76	52	44
Sampled area (km^2)	7,832	5,320	1,661	852
% Degraded area (<80% survival)	17±6	14±8	28±13	8±7
Sediment toxicity 1990				
Number of stations	84	34	26	24
Sampled area (km^2)	3,716	2,380	820	516
% Degraded area (<80% survival)	10±7	3±5	38±25	6±11
Sediment toxicity 1991				
Number of stations	88	42	26	20
Sampled area (km^2)	4,116	2,940	820	335
% Degraded area (<80% survival)	22±10	24±13	19±14	10±7
Sediment chemistry (1990-1991)				
Number of stations	202	96	59	47
Sampled area (km^2)	9,450	6,720	1,861	869
% Degraded area (>ERM)	7	4	16	13
Sediment chemistry (1990)				
Number of stations	104	48	332	24
Sampled area (km^2)	4,908	3,360	1,050	498
% Degraded area (>ERM)	8	4	23	5
Sediment chemistry (1991)				
Number of stations	98	48	27	23
Sampled area (km^2)	4,542	3,360	811	371
% Degraded area (>ERM)	6	4	8	24

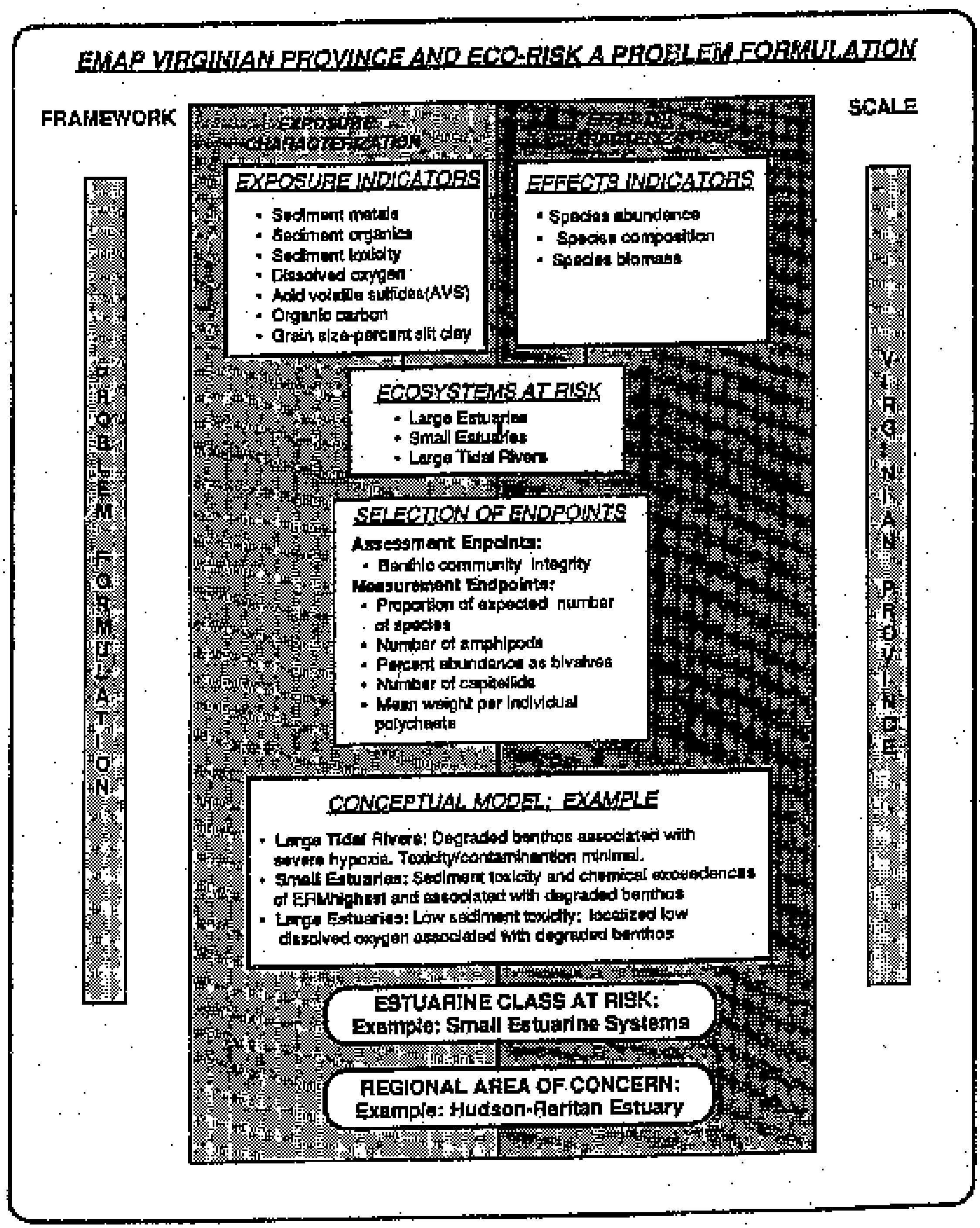

Figure 5-3. Contribution of EMAP data to problem formulation

Although the benthic index used in this case study appears to work well for distinguishing sites of differing environmental quality, other indices also may be effective. First, covariance among many of the candidate measurements was high, suggesting that several alternative combinations could produce comparable results. Second, index development was based on only 33 indicator testing sites that, although representative, did not represent all possible conditions. Third, the stepwise discriminate analysis may not have included important measurements of the benthic assemblage. Indicator development needs to be a flexible process: as other studies or the analysis of large data bases suggest increased confidence in selected measurements, they can be incorporated into the developing index through forced stepwise discriminate analysis.

5.3.2.2. Exposure

The case study characterized exposure by determining the areal distribution of each exposure indicator: low dissolved oxygen, sediment toxicity, and metals and organic contaminates in the sediments. Data for each exposure indicator, collected during the index period (July to September), were analyzed individually and then combined for the Virginian Province and for large estuaries, small estuarine systems, and large tidal rivers. Critical values were selected for each indicator: dissolved oxygen ≤2 ppm; sediment toxicity ≤80 percent control survival; and sediment chemistry values > Effects Range-Median (ER-M) (Long and Morgan, 1990). The case study did not include estimates of bioavailability based on total organic carbon and acid volatile sulfides or simultaneously extractable metals (Di Toro et al., 1991, 1992). Cumulative distribution functions were used to calculate the percent area for exposure indicator values. Note that it is not the intent of EMAP to characterize naturally occurring seasonal variability or to assess status for all seasons. Table 5-1 summarizes data for dissolved oxygen, sediment toxicity, and sediment chemistry for 1990 and 1991 individually and for 1990-1991 combined.

- *Virginian Province*: Bottom dissolved oxygen concentrations lower than 2.0 ppm occurred in 6±4 percent of the area of the province. The extent of area affected in 1990 was similar (7±6 percent) to that in 1991 (4±4 percent). Toxic sediments occurred in 17±6 percent of the estuarine area, and more estuarine area showed toxicity in 1991 (22±10 percent) than in 1990 (10±7 percent). Sediment contaminant concentrations exceeding the ER-M values of Long and Morgan (1990) were found in 7 percent of the estuarine area sampled over the 2 years; the extent of area exhibiting exceedances in each year was similar (8 percent in 1990, 6 percent in 1991).

- *Large Estuaries*: Low bottom dissolved oxygen occurred in 5±4 percent of the sampled area of large estuaries; the 2 years had similar estimates for affected area: 6±7 percent in 1990 and 4±5 percent in 1991. The amount of large estuarine area exhibiting toxic sediments was 14±8 percent. An eightfold difference occurred in the extent of toxic sediments between 1990 (3±5 percent) and 1991 (24±13 percent). The interannual difference in extent of sediment toxicity was not reflected in Long and Morgan exceedances in chemical concentrations. The 2-year and single-year estimates of area affected by contaminant exceedances were all 4 percent.

- *Small Estuaries*: For small estuaries, the area with low dissolved oxygen did not exceed 1 percent for the 2-year or either of the single-year samples. Conversely, toxic sediments were much more prevalent in small estuaries. Twenty-eight (±13) percent of the area exhibited toxic sediments over the 2 years. Nearly twice the sampled area was affected by toxic sediments in 1990 (38±25 percent) than in 1991 (19±14 percent). This pattern also was found for the extent of exceedances in contaminant concentrations, where 23 percent of the area in 1990 exhibited elevated contaminants compared with only 8 percent of the area in 1991. The estimate for the affected area in the 2-year composite was 16 percent.

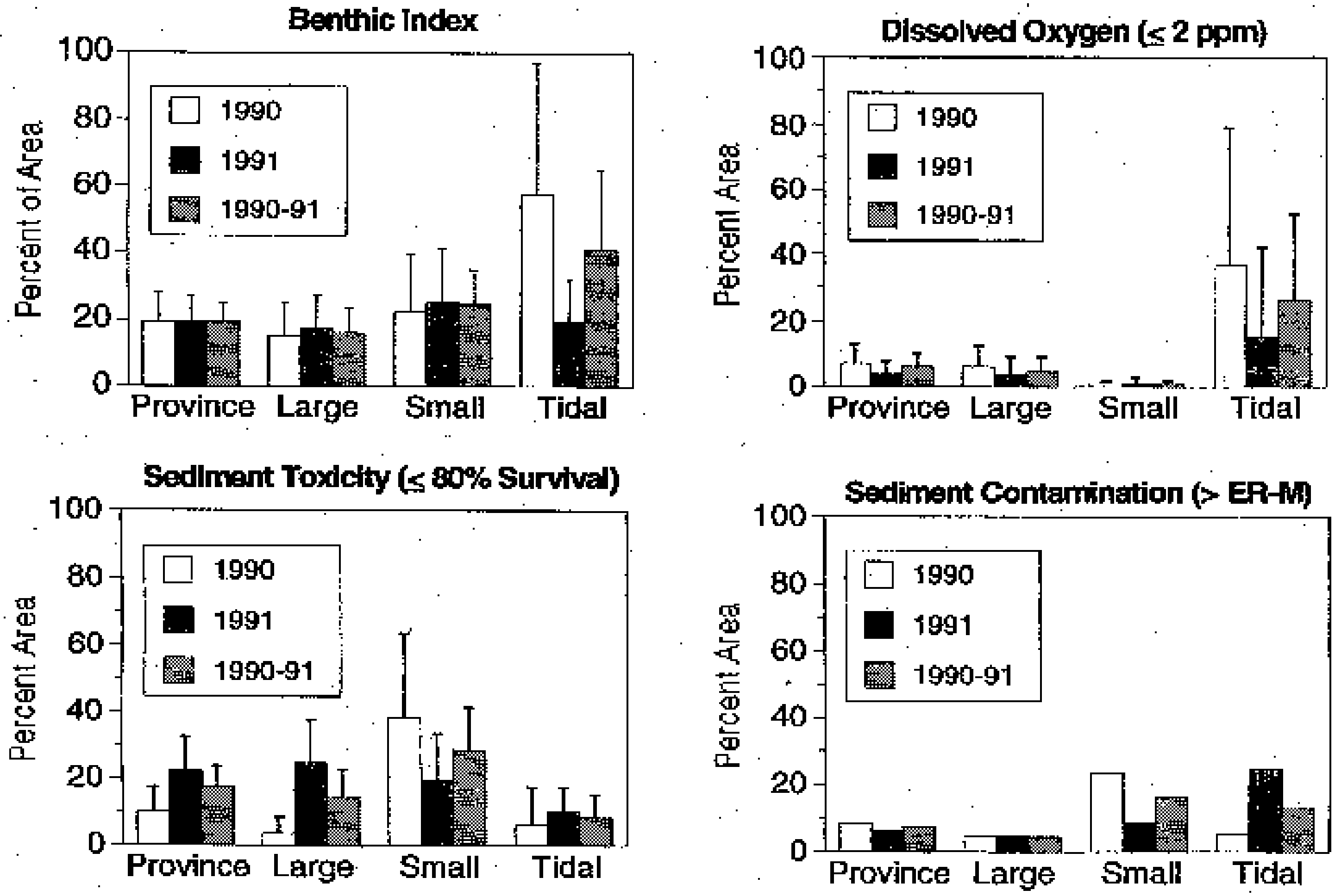

Figure 5-4. Summary of Virginian Province response and exposure indicator values for the entire province, large tidal rivers, large estuaries, and small estuarine systems for 1990 and 1991 data, individually and combined

- # *Large Tidal Rivers*: Low dissolved oxygen was most widespread in the large tidal rivers with 26±26 percent of the area exhibiting dissolved oxygen concentrations <2 ppm. For 1990, the extent of area with low dissolved oxygen was over two times the value for 1991 (37±42 percent vs. 15±27 percent). Toxic sediments occurred in 8±7 percent of the tidal river area over the 2 years; the affected area in either year did not surpass 10 percent. In contrast to small estuaries, the 1991 value for the percent area having elevated contaminant concentrations exceeded the 1990 value: 24 percent for 1991, as compared with 4 percent for 1990. Overall, 13 percent of the tidal river area was degraded relative to this indicator.

The percent area of low dissolved oxygen (<2 ppm) ranged from 1 percent (13 km^2) in the small estuarine systems to 4 to 6 percent (140 to 211 km^2) in large estuaries and 15 to 37 percent (56 to 159 km^2) for large tidal rivers. These data suggest that, based on percent area, low dissolved oxygen presents a greater problem in tidal rivers than in any other estuarine class (figure 5-4). However, when compared on the basis of absolute area, tidal rivers and large estuaries appear quite similar. In contrast, the percent area of sediment toxicity was consistently greater in small systems, 28 percent (465 km^2), than in large estuaries, 14 percent (745 km^2), or tidal rivers, 8 percent (68 km^2). However, when compared on the basis of absolute area, the area of sediment toxicity was almost twice as extensive in large estuaries than in small systems. The sediment chemistry data for 1990-1991 indicate that the small estuarine systems are at the greatest risk. However, this type of degradation does not show consistent distribution between the 2 years (table 5-2). These data reinforce the need to use the entire 4-year data set to minimize uncertainty in the description of estuarine condition.

5.3.2.3. Exposure-Response Associations

An important aspect of the conceptual model is the development of qualitative and quantitative associations or co-occurrences between exposure information and ecological effects information. Such associations lead to the development of hypotheses that can explain the observed changes in ecological responses and that can direct analyses in subsequent phases of the framework and further research. Because of the uncertainty inherent in this stage of the risk assessment process (Layard and Silvers, 1989), these hypotheses may not indicate causality. In fact, a definitive statement of causality is not a prerequisite for a risk assessment (U.S. EPA, 1992). Four areas of interest involve associations of benthic degradation with (1) low dissolved oxygen, (2) sediment toxicity, (3) both of the exposure indicators, and (4) neither of the exposure indicators. A separate analysis compares the co-occurrence of degraded benthos with the percent area for one or more sediment contaminants exceeding the ER-M values of Long and Morgan (1990). Table 5-2 presents these analyses, conducted for the Virginian Pr ovince, large estuaries, small estuarine systems, and tidal rivers.

- # *Virginian Province*: In the Virginian Province, 20 percent of the total area has degraded benthos. Of the 1,844 km^2 with degraded benthic condition, 17 percent co-occurs with sediment toxicity, 21 percent co-occurs with low dissolved oxygen, <1 percent have both, and 62 percent is not associated with either toxicity or low dissolved oxygen. These data suggest that low dissolved oxygen and sediment toxicity are almost equally associated with the area of degraded benthos in the province and together co-occur with 40 percent of the degraded area. The remaining 60 percent of degraded benthos is not associated with either exposure indicator. The percent area of degraded benthos that co-occurred with ER-M exceedances was 16 percent for 1990-1991 combined, 24 percent for 1990, and 7 percent for 1991.

Table 5-2. Indicator Associations: The Percent Area of Degraded Benthos Co-occurring With Low Dissolved Oxygen and Sediment Toxicity for EMAP Base Stations for 1990-1991[a]

Exposure Indicators	Estuarine Classes							
	Virginian Province		Large Estuaries		Small Estuaries		Tidal River	
	Area (km^2)	Percent	Area (km^2)	Percent	Area (km^2)	Percent	Area (km^2)	Percent
D.O. = <2.0 mg/L	380	21	210	20	13	3	158	45
Sedtox <80% survival	315	17	70	7	210	48	35	10
D.O. + sedtox	13	<1	0	0	13	3	0	0
Neither D.O. nor sedtox	1,136	62	770	73	207	47	159	45
Sediment chemistry (>ER-M)	295	16	70	7	197	50	28	8

[a]The co-occurrence of sediment chemistry and degraded benthos was calculated separately from sediment toxicity (sedtox) and dissolved oxygen (D.O.).

- # *Large Estuaries*: In large estuaries, 16 percent of the total area has degraded benthos: 7 percent co-occurs with sediment toxicity, 20 percent co-occurs with low dissolved oxygen, and there is no overlap in co-occurrence with both exposure indicators. These data indicate that 73 percent of the degraded benthos in large estuaries results from stressors other than sediment toxicity and low dissolved oxygen. Low dissolved oxygen did co-occur with degraded benthos in 20 percent of the area of large estuaries, principally in sections of Chesapeake Bay and Long Island Sound. The percent of degraded benthos that co-occurred with ER-M exceedances was 7 percent for 1990-1991.

- # *Small Estuaries*: Small estuarine systems present a somewhat different picture, with 24 percent of their total area exhibiting degraded benthos. Forty-eight percent of the area with degraded benthos co-occurs with sediment toxicity, 3 percent co-occurs with low dissolved oxygen, 3 percent co-occurs with both, and 47 percent of the area with degraded benthos is not associated with either low dissolved oxygen or sediment toxicity. These data illustrate a stronger relationship between sediment toxicity and degraded benthos in small estuarine systems. The data also suggest that dissolved oxygen is a less important factor. The percent degraded benthos associated with ER-M exceedances was 50 percent for 1990-1991 combined, 67 percent for 1990, and 25 percent for 1991.

- # *Tidal Rivers*: Tidal rivers have the highest areal extent of degraded benthos, 40 percent of the total class. In contrast to small estuaries, only 10 percent of the degraded benthic area co-occurs with sediment toxicity. However, 45 percent of the degraded benthos co-occurs with low dissolved oxygen, zero percent co-occurs with both exposure indicators, and the remaining 45 percent of the degraded benthic area in the tidal rivers is not associated with either exposure indicator. The percent degraded benthos for the tidal rivers associated with ER-M exceedances was 8 percent for 1990-1991 combined, 5 percent for 1990, and 20 percent for 1991.

The above approach represents one way of conducting analyses for associations. Other techniques are being explored (see Summers et al., 1993).

5.3.2.4. Estuarine Class Conceptual Models

In this case study, we have used only EMAP Virginian Province monitoring data for postulating potential risks for each estuarine class. Because only 2 years of data (1990 and 1991) are available, one must be cautious in their interpretation. The systematic, probabilistic sampling design includes 4 years of data collection to achieve complete coverage of the province and estuarine classes. Consequently, the areal estimates reported for both response and exposure indicators represent examples of how the data can be used and are not complete or accurate reflections of the power of the EMAP sampling design. However, even though designed around a 4-year sampling cycle, the estimates calculated from 2 years of data are representative of what would be expected for the whole province after 4 years of sampling. With additional years of data, the uncertainty will decrease, increasing the power to detect changes in areal extent.

In addition to being a monitoring program, EMAP is also a research program. Consequently, the choices of both response and exposure indicators must be viewed within the context of testable hypotheses. For example, data analyzed in this case study suggest that the algorithm used for the benthic index may require modification. However, since the benthic metrics (measurement endpoints) represent a consensus of what benthic ecologists deem important, variations in the index can be evaluated from the existing data bases. In fact, EMAP's indicator program is examining several other indices (Holland, 1990).

The exposure-response associations examined in this case study do not imply direct causality. For example, low dissolved oxygen and sediment toxicity are indicators of an aggregate of stressors from potentially a variety of causes and sources. Likewise, the sediment chemistry values, which were not normalized for bioavailability, provide only circumstantial evidence for ecological effects. The indicators used in this case study were never intended to assign causality. Rather, they provide preliminary information from a weight-of-evidence perspective. In conjunction with knowledge of other system properties (e.g., grain size, organic carbon, etc.), information from a weight-of-evidence perspective can identify potential problems.

The following summarizes our understanding, to date, regarding the potential problems in the Virginian Province and its three estuarine classes.

- *Virginian Province*: The assessment endpoint used in these analyses, benthic integrity/condition, was represented using a benthic index metric designed to discriminate "degraded" sites from reference sites. The data from 1990-1991 indicated that approximately 19 percent of the benthic area of the province was degraded according to the criteria established for the benthic index. Data from exposure indicators show that 6 percent of the province area experienced dissolved oxygen values <2 ppm, while 15 percent of the province area had toxic sediments. Seventeen percent of the degraded benthic area co-occurred with sediment toxicity (<80 percent control survival), while 20 percent co-occurred with low dissolved oxygen and 62 percent of the degraded benthic area was not associated with either indicator.

- *Large Estuaries*: For the most part, large estuarine systems are the downstream repositories of the stressor inputs entering from both the large tidal rivers and small estuarine systems. Approximately 16 percent of the area of large estuaries in the Virginian Province (1990-1991) exhibited degraded benthos. Not unexpectedly, the magnitude of sediment toxicity co-occurring with degraded benthos was only 7 percent. Twenty percent of the area of degraded benthos co-occurred with low dissolved oxygen. This area was restricted to the main stem of the Chesapeake Bay north of the Potomac River. In no areas of degraded benthos did low dissolved oxygen and sediment toxicity co-occur.

- *Small Estuaries*: The areal extent of degraded benthic communities in small systems for 1990-1991 was 24 percent. Only 3 percent of the area of the small estuarine systems with degraded benthos experienced hypoxic stress. In contrast, of the 24 percent of small estuarine area experiencing degraded benthos, 48 percent co-occurred with sediment toxicity. Approximately 50 percent of the area of small estuarine systems experiencing degraded benthos also had one or more sediment contaminant values exceeding the ER-M. Thus, a close correspondence exists in the annual patterns of sediment toxicity and sediment chemistry (>ER-M) in the small estuarine systems.

- *Tidal Rivers*: Just under one-half of the estuarine area in the large tidal rivers (40 percent) had degraded benthos in 1990-1991. Toxicity and hypoxic stressors rarely co-occurred at stations in the Virginian Province, including those in the large tidal river systems. Only 10 percent of the area with degraded benthos co-occurred with sediment toxicity, which was restricted spatially to the oligohaline headwaters (<0.5 ppt) of the Rappahannock, Delaware, and Hudson Rivers. In contrast, areas of low dissolved oxygen (45 percent) occurred primarily in the lower, mesohaline portions of the Potomac and Rappahannock Rivers. These data support current understanding of sediment contaminant distributions in urbanized waterways and of existing dissolved oxygen problems in the main stem of Chesapeake Bay. However, none of the five tidal rivers in the Virginian Province have areas of co-occurrence of both sediment toxicity and low dissolved oxygen.

5.3.2.5. Problem Formulation Summary

Of the three estuarine classes examined in this case study, large estuarine systems exhibited the lowest percent area of degraded benthos (16±7 percent), followed by the small estuarine systems (24±10 percent) and the tidal rivers (41±24 percent). Although areal extent of degradation is important, the spatial pattern (geographic distribution) of resource degradation is particularly important for identifying specific regional ecosystems at risk (figure 5-5). These data clearly suggest that much of the degradation of benthic resources is closely associated with the five tidal river systems and their associated small estuaries. The co-occurrence of exposure information on sediment chemistry, sediment toxicity, and dissolved oxygen was used to formulate hypotheses to suggest possible explanations for the observed spatial patterns of degraded benthos. Co-occurrence of low dissolved oxygen can be postulated as an explanation for 20 percent of the degraded benthos in large systems, but only for 3 percent of the degraded benthos in small systems and for more than 45 percent of the degraded benthos in tidal rivers. Conversely, co-occurrence of sediment toxicity can explain only 7 percent of the degraded benthos in large systems, 10 percent in tidal rivers, and 48 percent in small estuaries.

In addition, since hypoxia and toxicity co-occur infrequently (<5 percent), one might expect them to represent differing system and source characteristics. For example, toxicity was more prevalent in the lower salinity portions of these systems (mesohaline and oligohaline) than was low dissolved oxygen, suggesting a potential association with urban point sources in the upper reaches of estuaries. Chemistry data on the exceedances of ER-M values for one or more chemical contaminants support this interpretation. Analysis of these data suggests that toxicity problems within small estuarine systems are localized in small tidal rivers and small embayments bordered by heavily industrialized urban areas.

Hypoxia can result from municipal discharges in portions of tidal river systems independent of industrial discharges or from nutrient enrichment in those small systems deeper and more open to larger embayments. Poorly flushed small systems with high carbon loads characteristic of sewage discharges would lead to high sediment oxygen demand and hypoxia. Nonpoint runoff from agricultural land bordering small estuaries and coastal lagoons also may result in nutrient enrichment, subsequent algal blooms, and hypoxia. However, numerous and extensive studies focus on explanations for the low dissolved oxygen in the large estuaries, especially in the main stem of the Chesapeake Bay.

The results from this case study indicate that, of the three exposure indicators, sediment contamination and toxicity are the primary risks in small estuarine systems while low dissolved oxygen presents the primary risk in large systems and, particularly, the tidal rivers. These exposure data do not identify specific contaminant stressors, nor do the data imply that these are the only stressors of concern. This conclusion is supported by the fact that more than 50 percent of the area of degraded benthos was not associated with any of the exposure indicators used in this case study. Other unmeasured contaminants could cause the observed toxicity. While it is not the intent of this case study to conduct an evaluation of the EMAP sampling design and indicator programs, the analysis of data used in this case study has resulted in several observations on its utility in the ecological risk assessment process (comment box).

5.3.3. EMAP and Regional Risk Assessments

The data presented above could lead to the formulation of several hypotheses regarding ecological condition at the provincial (biogeographic) scale and potential causes of degraded conditions. For example, some hypotheses might address the relative effects of contaminants in small estuarine systems versus those due to low dissolved oxygen in large tidal rivers. The provincial scale

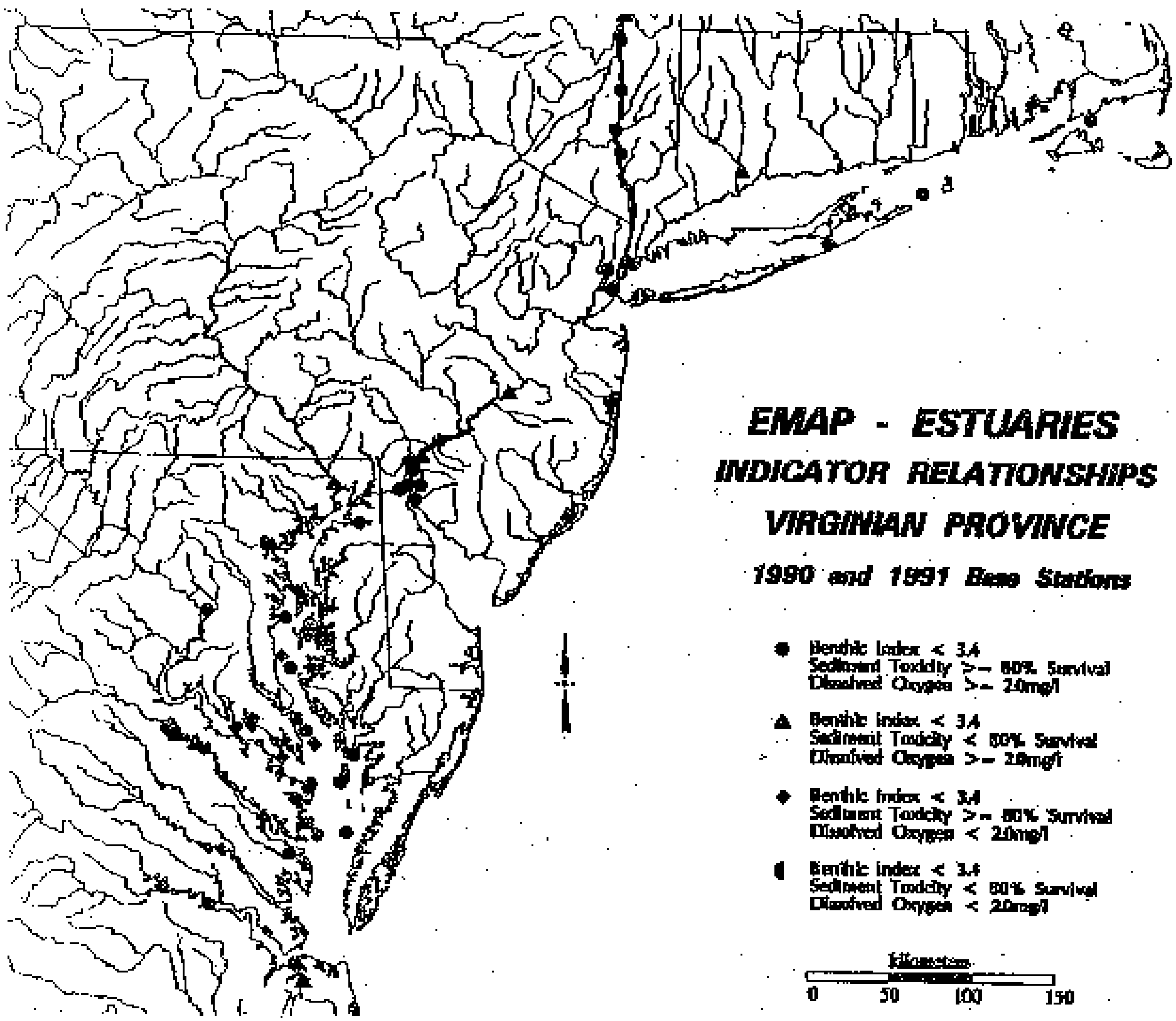

Figure 5-5. EMAP estuaries indicator relationships, 1990 and 1991 base stations

of EMAP sampling does not allow for adequate testing of hypotheses associating environmental exposure with ecological effect. Thus, finer-scale regional studies are necessary to refine and focus EMAP-generated hypotheses in a way that will lead to the development of more definitive cause-effect data. In addition to evaluating EMAP hypotheses, these assessments also should lead to more informed management decisions at the regional level. The following section presents an example of such an assessment.

Having demonstrated the use of EMAP province-scale information in the problem formulation phase of the risk assessment process, the next step would examine how the hypotheses developed at the province scale can be used to assess the regional risks to specific estuarine areas. The data and analyses presented above have focused on two types of information: (1) the distribution of benthic resources over large biogeographic areas (i.e., the Virginian Province) and (2) the relationship of those benthic resources to specific categories of exposure indicators. EMAP uses this information for characterizing and comparing the status of resources across provinces and within classes of estuaries. However, ecological resource data at the province scale have limited regulatory value; such data are not readily coupled to political boundaries and a control strategy via specific categories of stressors and defensible causal inferences. To optimize regulatory applicability, province-scale data must be placed within the context of regional assessments; that is, integrated into a risk-based decision framework that identifies the potential causal relationships between ecological resources and specific stressors and links the relationships to land-based activities amenable to source control.

EMAP data can identify the status of estuarine resources (represented in this case study by benthic resources) and, more importantly, the spatial patterns and extent of resource degradation within the province. These province-scale patterns can identify the types, spatial extent, and possible reasons for problems within various regional settings. Figure 5-5 illustrates the spatial distribution of degraded benthos within the Virginian Province after 2 years of sampling and the use of province-scale data to identify potential areas for regional assessments. Degradation generally is focused in the upper Chesapeake Bay, within the five tidal river systems and their associated small bays. These are areas of intense demographic pressure, extensive urban development, and the source of anthropogenic stress. Considerable benthic degradation occurs throughout the Hudson, East, and Raritan Rivers. This degradation is associated with sediment toxicity and elevated sediment chemistry values (figure 5-5). Data suggest that EMAP information can help identify regional areas of degraded resources and provide preliminary associations with exposure type. The probabilistic nature of the EMAP design also permits a determination of the relative magnitude of degradation, thereby focusing attention on areas with potentially the greatest problems. Using the Hudson-Raritan estuary and watershed as an example, the following sections briefly describe one approach for conducting a regional assessment that uses the EMAP design, indicator, and assessment concepts.

5.3.3.1. Regional Risk Assessment: Problem Formulation

While useful in identifying regional problem areas, EMAP province-scale data are not collected in sufficient detail for conducting a complete regional risk assessment. Although extant local monitoring data are usually available, they often are heterogeneous relative to spatial, temporal, and ecological scale and methodologies (e.g., type of sampling gear, analytical methods, etc.). Within the Hudson-Raritan basin, decades of monitoring data are available from NOAA, states, and more recently the Harbor Estuary Program (HEP). However, each of these programs has its own problem-oriented objectives and sampling and analysis goals. This heterogeneity in objectives makes it difficult, if not impossible, to satisfy the information needs of problem formulation and fully characterize the type and spatial extent of the ecological problems at a regional scale.

The first step, then, in the regional risk assessment involves revisiting the problem formulation phase of the risk assessment process to characterize the spatial extent of degraded resources and associated measures of exposure. Data for this purpose can be acquired through (1) an appropriately scaled monitoring program employing a random sampling design (e.g., EPA Region II, R-EMAP); (2) selection of the appropriate response, exposure, and habitat indicators to characterize the spatial extent of ecological problems and associated exposures; (3) the incorporation of extant data, where possible, into a probabilistic sampling design analogous to that used by EMAP; or (4) through a combination of all three approaches. The conduct of problem formulation at the regional scale will provide a detailed description and spatial representation of the types, magnitude, spatial distribution, and areal extent of ecological problems. These ecological effects can then be associated more closely with specific exposure and habitat indicators and stressors, leading to the development of one or more conceptual models for the region or specific watershed within the region. Currently, ORD, in cooperation with EPA Region II, is conducting a Regional-EMAP project in the Hudson-Raritan estuary to develop just such a series of conceptual models for various areas within the estuary.

5.3.3.2. Regional Risk Assessment: Analysis Phase

The analysis phase of the ecological risk assessment process involves the development of detailed models describing the spatial and temporal patterns of exposure and stressor-response models that illustrate the change in status of ecological response as a function of incremental changes in exposure. Monitoring programs may collect some types of data that are relevant to a detailed analysis of ecological risks; however, they do not normally collect the full spectrum of necessary data, nor do monitoring data provide the necessary uniform spatial coverage for the area of concern. Within a regional setting like the Hudson-Raritan, where sediment toxicity and contaminated sediments are known to be associated with degraded benthic resources, the risk assessor would likely synthesize extant data from the ORD research laboratories, Region II, HEP, NOAA, states, private sector, etc., to develop the causal relationships necessary to fully characterize the regional risks.

Extant data for this area can prove particularly important in identifying possible causes for observed resource degradation. For example, there is a history of PCB contamination in the Hudson River, dioxins in the Raritan River, petroleum contamination in the Arthur Kill River, and organic enrichment in Jamaica Bay. In addition, during the last several years NOAA has synthesized data on benthic community structure, sediment toxicity, and metal and organic contaminants in sediments and water in this area. Although not sampled probabilistically, these data help identify spatial patterns of problems and their potential causes in various sections of the estuary. A regional risk assessment can use these data, along with laboratory toxicity information and measures of contaminant bioavailability, to develop evidence needed for postulating causal inferences for the region as a whole or for a specific watershed. The causal relationships may be quantitative or inferential, relying on weight-of-evidence and professional judgment.

The contribution of the EMAP design to the Hudson-Raritan basin study, conducted by EPA Region II, will significantly strengthen inferences of risk within this watershed (National Governors Association, 1993). In addition, this R-EMAP project also will examine methods for incorporating extant data into the probabilistic EMAP design, further enhancing its utility. Most likely, monitoring data alone will prove insufficient for establishing the causal relationships necessary for developing a complete risk assessment. Nevertheless, the intent is to develop multiple, converging lines of evidence for linking observed ecological effects to one or more specific stressors or to stressor categories that are amenable to remediation. The extant data in the Hudson-Raritan basin suggest that different stressor-response relationships may emerge for different watersheds. This conclusion would lead to different source control management strategies for each watershed.

5.3.3.3. Regional Risk Assessment: Risk Characterization

The risk characterization phase of the framework describes three methods for integrating exposure and effects information into a statement of the likelihood of risk with associated uncertainties: point comparisons, distributional comparisons, and modeling. Depending on the type of data, any one or a combination of these approaches can be used with the types of monitoring data presented here. GIS and landscape methods can provide initial descriptions of risks to specific watersheds. These methods can describe the spatial relationships and distribution of response, exposure, and habitat indicators (stressor-specific whenever possible). These descriptions can then be overlaid with landscape information on hydrologic features (e.g., transport and fate) in the surrounding watershed. Descriptive approaches, using GIS and landscape methods, can integrate field data describing the spatial extent, magnitude, and degree of association between response and exposure indicators. However, descriptive approaches do not establish functional exposure-response relationships. Establishment of functional relationships requires the decomposition of measurements of "aggregate exposure" (e.g., sediment toxicity-related bioeffects from multiple stressors) into specific stressors using diagnostic biomarkers, fractionation protocols, and laboratory ecotoxicity tests.

For example, overlays of response and exposure indicators indicate that there is a high degree of co-occurrence of benthic community degradation with sediment toxicity and specific sediment organic contaminants (e.g., dioxins and dibenzofurans) in the Raritan River. This example suggests the potential for a strong causal relationship between specific stressors and ecological effects. Literature data, additional in situ field testing along a gradient, or laboratory testing can evaluate the hypothesis. As a clearer picture of the specific stressors emerges, GIS and landscape methods can integrate (1) information on the spatial distribution of specific contaminants, (2) areas of degraded benthos, (3) information on the discharges from land-based activities, and (4) hydrologic information from the surrounding watersheds. Using available data and converging lines of evidence, a series of inferences can be developed regarding causal associations from response to exposure to stressors to sources. Supporting these initial inferences requires additional analyses such as site-specific studies on organism-residue relationships, contaminant "spiked" laboratory sediment-residue and toxicity analyses, and site-specific field studies using natural contaminant gradients. Together, these studies would focus on quantifying functional and causal relationships and the uncertainties associated with each phase of this process.

In summary, spatial models describing response-exposure-stressor-hydrologic relationships can be coupled with landscape models describing specific watershed activities that are sources of anthropogenic inputs. The establishment of the appropriate causal relationships between sources and effects provides the basis for the manager to institute appropriate control strategies. Existing local compliance (e.g., NPDES, states, municipalities) and watershed assessment (R-EMAP, EMAP, NS&T) monitoring programs can evaluate the effectiveness of the control strategy.

Comments on Problem Formulation, Conceptual Model Development, and Regional Risk Assessments

General reviewer comments:

- *The case study's introduction and the background do a good job of setting the stage for the problem formulation and of explaining the benefits and limitations of the EMAP program. The authors refer to the use of EMAP in this fashion as a "weight-of-evidence" approach. Perhaps it would be more accurate to call it a screening approach, because "weight-of-evidence" has a toxicological interpretation that implies real knowledge of cause and effect for a stressor and organisms. In using the term "weight-of-evidence," EMAP is suggesting such a relationship.*

Comments on Problem Formulation, Conceptual Model Development, and Regional Risk Assessments (continued)

- *The percent co-occurrence of degraded benthos with low dissolved oxygen does not give the percent of degradation that can be attributed to low oxygen. As stated, these co-occurrence data represent a contingency table that tests association. Consider the large estuaries where 7 percent of degraded benthos is associated with sediment toxicity, despite the fact that 14 percent of the estuaries have sediment toxicity. The conclusion is (if significant) that sediment toxicity tends to be associated with undegraded benthos. For oxygen, the respective figures are 20 percent and 5 percent; hence, low oxygen appears to be quite strongly associated with degraded benthos.*

  There are a number of ways of analyzing for associations in such data. If one can assume that samples are independent, then a log-linear model of frequency data might be appropriate. In this case, sample size permitting, there could be four levels: riverine type, benthos condition (degraded vs. undegraded), sediment toxicity, and ER-M exceedance. Such an analysis would determine differences among riverine types in various conditions, associations of exposure measures with effect measures, and associations among the different exposures.

- *The section on regional risk assessment refers to the association of degraded areas with areas of "intense demographic pressure, extensive urban development, and the source of anthropogenic stress." Should these be considered as part of the exposure characterization? Could sampling data be further stratified by stressed areas within waterways and by stressed and unstressed waterways?*

Authors' comments:

<u>EMAP Sampling Design</u>

Strengths of the case study include:

- *Quantifies areal extent of indicator values.*
- *Describes the spatial patterns and distribution of ecological resources and associated habitat and exposure indicators.*
- *Permits the estimation of uncertainties for indicator values.*
- *Quantifies postremediation changes in areal extent of resources and exposures.*
- *Scalable to regions and specific sites (e.g., bays, estuaries).*

Comments on Problem Formulation (Continued)

Limitations include:

- *Limiting sampling to index period (e.g., once per year) fails to address seasonality and episodic events.*
- *Sampling design currently does not capture local spatial scale and short-term temporal scale events.*
- *Incorporation of nonprobabilistic extant data with the EMAP's probabilistic sampling design is currently not feasible and is a major limitation for risk assessment applications.*

EMAP Indicators

Strengths of the case study include:

- *Sites of exposure and habitat indicators are measured simultaneously with response indicator.*
- *Response indicator is hierarchical in design, with clear links between assessment endpoints, measurement endpoints, and metrics.*
- *Habitat indicators are directly related, facilitating the interpretation of response and exposure indicator information.*

Limitations include:

- *Currently, EMAP has no response or exposure indicators for nutrient or carbon enrichment (eutrophication).*
- *Response indicators have been developed and applied only for benthic resources.*
- *Exposure indicators for physical stressors are lacking.*
- *There is currently no systematic program for validating existing indicators.*
- *Accurate measures of bioavailability are needed for interpreting contaminant exposure indicators.*
- *The benthic index metric, sediment toxicity, and bioavailability indicators require evaluation, validation, and revision.*

Comments on Problem Formulation (Continued)

General comment:

- ! *A critically important element of the program, the research component of EMAP, has not received adequate emphasis. While representing an excellent start at characterizing the status of resources in the Virginian Province, the EMAP indicator program will require revisions based on examination of 1990-1991 data. Specifically, the benthic index metric needs to be evaluated and potentially revised to include ecologically relevant measures of habitat characteristics, successional status, or functional attributes. The exposure indicators, sediment toxicity and sediment chemistry, should be reexamined from the perspective of bioavailability, provided that the toxicity tests are an accurate surrogate for community exposure and that exposure is accurately coupled with biologically relevant measures of contaminant availability. Currently, the absence of quantitative functional relationships between response and exposure indicators limits their predictive value in ecological risk assessments. However, these indicators can contribute to a qualitative weight-of-evidence approach to understanding the status and condition of ecological resources.*

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